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NOTES AND LECTURES FROM SANITARY ENGINEERING TRAINING COURSE IN STREAM SANITATION PRACTICES

ENVIRONMENTAL HEALTH CENTER
CINCINNATI, OHIO

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# Notes and Lectures
from
Sanitary Engineering Training Course

Cincinnati, Ohio

March 15 - April 2, 1948

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NOTES AND LECTURES

from

SANITARY ENGINEERING TRAINING COURSE

in

STREAM SANITATION PRACTICES

Conducted at the

ENVIRONMENTAL HEALTH CENTER

PUBLIC HEALTH SERVICE

Cincinnati, Ohio

March 15 - April 2, 1948

Public Health Service
DIV. OF SANITARY ENGINEERING SERVICES
Reference Room

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Bactericidal Efficiency of Quaternary Ammonium Compounds

(C. W. Chambers)

I. Interest widespread and increasing. Approximately 100,000 formula combination possible.

II. Laboratory test methods

  A. Phenol Coefficient. (1)

    1. Based on 5, 10, and 15 minute exposure intervals.

    2. The test is made in a solution consisting of 90% germicide water mixture plus 10% broth culture.

      (a) Inhibitory due to presence of foreign organic material.

      (b) Not medium (dish water etc) used in practical application.

      (c) Small test volume limits amount of chemical testing that can be done.

    3. Result is in terms of relative rather than actual potency.

  B. Cincinnati Station Method (large volume)

    (1) Uses 500 ml volume.

    (2) Natural tap or variable waters.

    (3) 1, 2 and 5 minute exposure intervals.

    (4) Foreign matter a negligible factor.

    (5) Method reproducible in parallel tests.

III. Field Test Methods

  A. Not satisfactory at the present time.

    (1) Tests for p.p.m. may bear little relationship to the actual effective germicide present due to the tests measuring non-bactericidal addition products.

    (2) In the case of many Q.A.C. the active agent is not specifically subject to quantitative analysis.

(3) What is badly needed is a field test which will provide a reading in terms of bactericidal activity, regardless of what the total concentration of Q.A.C. (in p.p.m.) may be..

IV. Laboratory Findings

A. Factors which, when varied, alter the effectiveness of Q.A.C.

(1) pH

(2) Temperature

(3) Different natural waters.

(a) Mineral content.

(b) There are factors other than mineral content or in conjuntion with it, their identity as yet, has not been established, which in extremely minute quantities appear to exert an influence which is enormous when compared with the effect of mineral content alone.

(4) Detergents - some interfere seriously.

(5) Soap and organic material inactivate the bactericide.

V. Application of preceding findings

A. Precautions which should be observed.

(1) Determine by means of adequate reproducible laboratory tests how any compounds which you may anticipate approving behave in the water in which they will be used.

(2) The swab test is subject to too much variation in the manner of its application to be considered satisfactory for this purpose.

(3) Keep soap, detergents, and other foreign material out of sterilizing rinses.

(4) Having tested a given commercial product containing a specified percentage of a certain active agent keep in mind that it does not necessarily follow that other products using the same active agent will be equally effective when used in comparable concentrations.

(1) Kolmer and Boerner, Approved Laboratory Technic (Fourth Edition) page 515.

Bactericidal Properties of Chloramines and Free Chlorine in Water

(C. T. Butterfield)

When chlorine was first used for the disinfection of drinking water supplies it was generally believed that there was a fixed amount of chlorine which would sterilize all waters. Studies at that time were pointed toward the determination of this optimum dose. During the years that followed it became known that enough chlorine had to be added, (1) to satisfy the chlorine demand of the water, and (2) to provide a residual chlorine concentration which would insure disinfection. Interest then centered, for a number of years, on the determination of residual chlorine and of the standard chlorine residual which would insure proper disinfection of the water under any condition. The bactericidal results, which were obtained using a standard residual chlorine, were inconsistent and the disinfection in many instances were unsatisfactory.

The development of chloramine treatment and break-point chlorination for the prevention and/or destruction of tastes and odors led to a recognition of many factors affecting the disinfection of water with chlorine, as follows: (1) free chlorine is a much more potent disinfecting agent than chloramines, (2) the standard orthotolidine test did not differentiate between free chlorine and chloramine residuals, (3) both pH and temperature affected the bactericidal efficiency of free chlorine and chloramine, and (4) the habit of thinking of residual chlorine without differentiating between chloramine and free chlorine was without doubt responsible for the inconsistencies in the results obtained, differences of opinion, and failures to establish satisfactory standard residuals.

Prior to the development of adequate tests for the separate determination of free chlorine and chloramine, studies were made in our laboratory of the bactericidal properties of each one of these two substances under carefully controlled conditions, so that there could be no question concerning the purity of the bactericidal agents acting. That is, tests were made with free chlorine with all traces of chloramine excluded, and in tests with chloramine no free chlorine was present. These studies were made at pH values ranging from 6.5 to 10.7 and in two temperature ranges of 2° to 5°C., and 20° to 25°C. The bacteria used for the tests were not limited to organisms of the coliform group. Escherichia coli and Aerobacter aerogenes, but included also strains of Pseudomonas pyocyanene, Eberthella typhose, and Shigella dysenteriae. The shigella or dysentery group, which might be considered the most important from a sanitation viewpoint, included a number of varieties of shiga, Flexner, Boyd 88 and sonnei strains.

These studies have been reported in detail in Public Health Reports and are available in Reprints, N. 2530, "Influence of pH and Temperature on the Survival of Coliforms and Enteric Pathogens when Exposed to Free Chlorine," and No. 2692, "Influence of pH and Temperature on the Survival of Coliforms and Enteric Pathogens when Exposed to Chloramine", to those who may wish to make a more thorough study of the results.

In the following discussion these results are presented; (1) in summary, with a view to making a practical application of the more important features, (2) contrasting the relative efficiency of free chlorine and chloramine in water disinfection processes, and (3) proposing for consideration certain minimum standards with the supporting data for the proposed safe residuals under the various conditions.

In Figure 1, for purpose of illustration, an ideal diagrammatic residual chlorine curve in the presence of 0.9 ppm of free ammonia, as nitrogen, is presented. It is noted that with 0.9 ppm of ammonia nitrogen initially present (1) there is no change in the ammonia nitrogen content until 4.5 ppm of chlorine have been added, (chlorine: amonia-nitrogen ratio of about 5 to 1), (2) during this period the orthotolidin (O.T.) residual chlorine content is the same as the amount of chlorine added, and (3) all of the residual chlorine is present as chloramine. When this point (popularly called the "hump") is reached, added amounts of chlorine results in corresponding decreases of, (1) O.T. residual chlorine, and (2) ammonia-nitrogen, until when about 9.0 ppm have been added, the residual chlorine content and the ammonia-nitrogen content will be zero.* During this period of decline the residual chlorine present is chloramine. Increments of chlorine after this

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(*) For a fuller discussion of the chemistry of these reactions, the reader is referred to a paper, "Some Chemical Aspects of the Ammonia-Chlorine Treatment of Water", by W. A. Moore, S. Mergregian, and C. C. Ruchhoft, Jour. Am. Water Works Association, Vol. 35, No. 10, October 1943, pp 1329-1343.

zero point (referred to as "break-point") produce corresponding increases in residual chlorine and this residual chlorine is free chlorine. If the amounts of chlorine added are so adjusted that this zero point is achieved, then there is neither free chlorine nor chloramine present and the water is free entirely of any bactericidal properties. This would be the case in the instance cited, though 9.0 ppm of chlorine had been added to the water. This figure is presented primarily to demonstrate that exactly at break-point, (1) no free chlorine or chloramine remain, and (2) no bactericidal action occurs. To support the latter statement, though this exact endpoint in the reaction is difficult to attain, it has occurred in several of our tests and definite bacterial testimony obtain that bactericidal action does not occur at this point. Post break-point chlorine is free chlorine which may be demonstrated by the test procedure indicated, or by any one of several tests which have been developed by the chemists.

In Figure 2, the influence of temperature on the bactericidal properties of free chlorine and chloramine is illustrated and the relative efficiency of free chlorine and chloramine is contrasted. The points recorded for chloramine are based, on results obtained after 60 minutes of exposure at the temperature given, and the results for free chlorine are after only 20 minutes of exposure. It is noted that under these conditions:

(1) at pH 7.0 with chloramine, 0.6 ppm produces a 100 per cent kill at 22°C. and 1.5 ppm is required to produce the same result at 4.°C.

(2) At pH 8.5 with chloramine, 1.2 and 1.8 ppm were required to produce a 100 per cent kill at 22°C and 4°C. respectively:

(3) Comparisons cannot be made for chloramine at pH ranges above 8.5 as 2.0 ppm of chloramine was the maximum amount used in these tests and 2.0 ppm of chloramine did not produce 100 per cent kill at 4°C in 60 minutes,(with 120 minutes of exposure 1.8 ppm of chloramine produced a 100 per cent kill at 4°C.)

(4) With free chlorine at pH ranges of 7.0 and 8.5 the bactericidal properties are not affected materially by the low temperature as 100 per cent kills were obtained in 20 minutes with 0.03 to 0.06 ppm of free chlorine.

(5) With free chlorine at higher pH ranges, 9.8 and particularly 10.7, the lower temperature markedly affects the bactericidal efficiency; thus:

(a) At pH 9.8 0.4 ppm was required at 4°C and only 0.06 ppm at 22°C.

(b) At pH 10.7, 1.0 ppm was required, and only 0.3 ppm at 22°C.

In Figure 3 the relative efficiency of free chlorine and chloramine is contrasted by results obtained at 20° to 25°C., after various periods of exposure. In general the lines of 100 per cent kill intergrade very nicely for the various time intervals. With both free chlorine and chloramine plotted to the same scale direct comparisons may be made. It is sufficient perhaps to note that under the same conditions of test the bactericidal efficiency of chloramine with 120 minutes of exposure does not quite equal the efficiency of free chlorine with one-minute of exposure. Comparisons of the amounts of free chlorine and chloramine required to obtain 100 per cent kills in the same time interval are not possible for periods of less than five minutes as chloramine in the concentrations used did not produce 100 per cent kills in less than five minutes. As a matter of fact, results with chloramine were not consistent for periods of less than about 20 minutes. For instance, the respective amounts of free chlorine and chloramine required to produce 100 per cent kills, were, after 20 minutes, at pH 7.0, 0.04 ppm and 1.2 ppm (1 - 30), at pH 8.5 0.07 ppm and 1.8 ppm (1-26): after 60 minutes at pH 7.0, 0.04 ppm and 0.6 ppm (1-15), at pH 8.5, 0.05 ppm and 1.2 ppm (1-24), at pH 9.5, 0.06 ppm and 1.5 ppm (1-25), at pH 10.7, 0.03 ppm and 1.8 ppm (1-60). These results indicate that if 100 per cent kills are to be obtained in the same exposure times, then 15 to 60 times (average 30) or 26 if the two extremes 15 and 60 are omitted from the average, as much chloramine must be used as compared with free chlorine. Thus, it can be conservatively stated that, (1) chloramines are much less efficient as bactericidal agents than free chlorine, (2) to obtain 100 per cent kills under the same conditions, (a) with the same amounts of free chlorine or chloramine will require at least a 100 times longer exposure period for the chloramine, and (b) with the same period of exposure will require at least 25 times as much chloramine as free chlorine.

It is believed that this evidence on the influence of pH and temperature on the effectiveness of free chlorine and chloramines is sufficiently applicable to plant operation with natural waters to justify proposing suggested minimum standards for water chlorination. In formulating such proposals, however, the influence of both pH and temperature must be kept in mind. For this purpose Figure 4 is presented to show the effect of low temperature. It is noted that at high pH ranges, 10 to 11, two ppm of chloramine for two hours, or one ppm of free chlorine for 10 minutes is not sufficient to produce a 100 per cent kill. In the case of free chlorine, if the exposure period be increased to 60 minutes, then 0.3 ppm is effective at pH 10.7. Similarly, with chloramine, if the time of exposure were increased to four hours, undoubtedly 100 per cent kills would be obtained at this high pH. However, at pH ranges above 9 and particularly above 10, for contact periods of more than one hour, the hydroxyl ions show a very marked bactericidal action and the results cannot be ascribed to residual chlorine alone. This has been the subject of an earlier report, (Relative Resistance of Coliform Organisms and Certain Enteric Pathogens to Excess-Line Treatment"), by Elsie Wattie and C. W. Chambers, Journal Am. Water Works Association, 35: 709 (1943).

In figure 5, utilizing all of the data obtained at 20° to 25°C., for 60-minute exposures to chloramine and 10-minute exposures to free chlorine, with all strains of coli, aerogenes, pyocyaneae, typhose and dysenteriae tested straight lines* have been drawn to indicate the general trend of the 100 per cent kill for all of the strains tested. In addition there have been indicated the proposed minimum safe residuals for chloramine after 60 minutes of contact between organism and bactericidal agent, and for free chlorine after 10 minutes of such contact. In setting up such proposed minimum safe residuals, it is essential to provide a liberal factor of safety to allow for varying conditions such as the; (1) frequency with which residual tests are made, (2) adequacy of operators in training and experience, (3) reliability of the chlorine feeding method, (4) variations in flow and in the chlorine demand of the water, etc.

For free chlorine with waters of pH 6.0 to 8.0 a safe residual after 10 minutes would be not less than 0.2 ppm; at pH 8.0 to 9.0 at least 0.4 ppm; at pH 9.0 to 10.0 at least 0.8 ppm; and at pH ranges of 10.0 and above , more than 1.0 ppm, or preferably periods of contact of four hours. The better solution for the high pH problem, when possible to do so, would be to reduce the pH below 9.0.

(*) This contrast has also been made using chloramine results after 120 minutes and free chlorine after 20 minutes of exposure without materially altering the trends of the lines of 100 per cent kills, particularly if the low temperature results are included. Consequently, it is believed that the proposed minimums should be the same for these periods.

For chloramine, with waters of pH 6.0 to 7.0, a safe residual after 60 minutes of contact would be not less than 1.0 ppm; at pH 7.0 to 8.0 at least 1.5 ppm; at pH 8.0 to 9.0 not less than 1.8 ppm; and at pH ranges above 9.0 an undetermined amount of chloramine. Consequently, with chloramine, for pH ranges of 9.0 and above it would be better to reduce the pH below 9.0 when possible to do so, or to extend the contact time to four hours, as in general 1.8 ppm of chloramine will produce a 100 per cent kill in two hours of contact at pH 9.5 at either of the temperature ranges investigated.

In the application of these proposed standards, if there is any doubt as to the nature of the bactericidal agent acting, (that is, whether it is free chlorine or chloramine, as determined by the break-point procedure outlined, or by any of the chemical tests), then the safe procedure to follow is to assume that all of the residual chlorine present is chloramine and apply the chloramine standard. Detailed and complete data forming the basis for these proposed standards and observations, will be found in the tables of the references cited. The superiority of free chlorine as a bactericidal agent when compared with chloramine is quite evident. In addition, it is believed that the proper use of free chlorine will eliminate many taste and odor problems.

In general it can be statedthat the primary factors governing the bactericidal efficiency of both free chlorine and chloramine are:

(1) the time of contact of organism and bactericidal agent: -

the longer the time, the more effective sterilization.

(2) the temperature of the water in which the contact is made: - the lower the temperature, the less effective the sterilization.

(3) The pH of the water in which contact is made: - the higher the pH, the less effective the sterilization. Thus, when the combination of high pH and low temperatures is encountered the poorest results are to be anticipated.

Comparing the relative efficiency of free chlorine and chloramine it can be stated:

(1) under the most favorable conditions, i.e., at pH 7.0 and a water temperature of 20° to 25°C., 100 per cent kills cannot be obtained with chloramine residuals of about 1.2 ppm in 10 minutes, but may be obtained with 20 minutes of content. Under similar conditions with free chlorine 100 per cent kills are obtained with 0.04 ppm residuals in one minute of contact.

(2) to obtain a 100 per cent kill with the same contact period requires about 25 times as much chloramine as free chlorine.

(3) to obtain a 100 per cent kill using the same amounts of residual chloramine and free chlorine, requires approximately 100 times the contact period for chloramine.

Twelve slides from reprints 2530 and 2692 were discussed.

A Brief Review of the Studies of the Biological Oxidation of Sewage and Organic Wastes

(Effective Bacteria in Sewage Purification)

(C. T. Butterfield)

The research studies of the activated sludge and the trickling filter processes of sewage purification conducted at the Cincinnati Laboratory for a period of about ten years, starting in 1932, included investigations of the basic fundamental mechanisms of the processes in addition to practical observation on the efficiency of operation. As a result of these investigations the following observations have been made:

1 - Zoogleal masses were observed in large numbers in every specimen of good activated sludge or trickling filter growth, examined. This conforms with earlier reports in the literature.

2 - Zooglea-forming bacteria were isolated in pure culture from the sludge of both processes.

3 - These bacteria in pure culture, both in synthetic media and in natural sewage, produced floc and sludge which simulated normal sludge.

4 - These pure culture flocs were shown to remove during a 3-hour incubation period as much as 90 percent of the carbonaceous oxidizable material present in polluted water.

5 - The pure culture flocs on examination contained only the

same zoogleal bacteria which had used for seed. Thus Koch's Postulates, as applied to oxidation processes, had been fulfilled.

Further detailed studies with these pure cultures showed that:

1 - Approximately 50 percent of the 5-day B.O.D. of sewage is oxidized by these pure cultures sludges during the first 5-hours of aeration.

2 - 80 percent is oxidized in 24 hours.

3 - At any interval, approximately 25 percent of the oxidizable material present is held in the zoogleal matrix of the sludge.

Additional inquiry into the fundamental bacteriological processes of biochemical oxidation was made along several lines. For instance, it was found that when the initial bacterial population was limited to 500,000, or less, per ml (as is practically always the case in oxygen requirement determination by the excess oxygen dilution method), the incidence of measurable oxidation is slow, varying with the initial number of organisms present, and oxidation proceeds in an orderly fashion with the maximum hourly rate attained only after several hours of incubation. This is in keeping with the orderly progress of bacterial growth.

When the initial bacterial population is greater (3,000,000 to 10,000,000, or more, per ml), the incidence of measurable oxidation is very rapid, the maximum hourly rate of oxidation is attained very early in the life of the sample (from at once to 4 hours) and thereafter the hourly rate of oxidation decreases quite rapidly.

This diminution in rate of oxidation, after the hourly peak rate has been reached, would be presumed to be caused by the decrease in the amount of oxidizable food available per bacterial unit (i.e., a situation is reached soon when this amount of food is no longer sufficient to stimulate growth and reproduction but is sufficient only to maintain life.) The results indicate that the amount of oxygen required for bacterial life processes under such limited nutritional conditions is very small indeed (less than 0.01 mg of oxygen per million bacteria per day).

With all initial bacterial concentrations tried, regardless of the time required for the incidence of measurable oxidation, and regardless of the time of occurrence of the maximum hourly rate of oxidation, the total oxygen requirement after a 24-hour or longer period was approximately the same in all cases with the same concentrations of food and the same bacterial species acting. It appears logical to assume that, if these final bacterial populations had been reduced below their limiting number, renewed growth with its consequent increase in oxidation would have ensued. In fact, in biological processes such bacterial reductions or removals have been shown to be essential for the completion of both natural and artificial methods of purification.

While the bacterial maxima observed in the second series of experiments, using an aerogenes culture which tended to form flocs, were considerably greater than those obtained in the preceding experiment using a nonclumping strain of aerogenes, the increased bacterial numbers secured in this manner did not approach the huge concentrations of bacteria present in activated sludge. In pure culture activated sludge with a suspended solids content of about 1,000 p.p.m. (dry weight) the bacterial content is at least 10,000,000,000 per ml. It is suggested, therefore, that this phenomenon of the initial acceleration of oxidation induced by increased initial numbers of bacteria is the explanation of the very greatly increased rates of oxidations reported for activated sludge, (5) (6).

Because, in these experiments, when the initial numbers of bacteria were increased from a few thousands up to 10 millions per ml, the portion of the total oxygen requirement satisfied during the first few hours was very greatly increased, it would be reasonable to conclude that in an activated sludge with the concentration of bacteria present at the start of aeration raised to 10 billions or more per ml, a still larger proportion of the total oxygen requirement would be satisfied during the first few hours.

From a bacteriological viewpoint such deductions appear logical, for in the activated sludge method of sewage purification the continuance of the process, namely sustained growth and oxidation, is dependent on: (1) a continuous addition of sufficient, available bacterial food (by an inflow of suitable sewage of appropriate strength), (2) a continuous reduction of the excess bacterial population (by the removal of accumulated sludge in excess of required volumes), and (3) the withdrawal of by-products detrimental to bacterial growth (by the discharge of effluent).

Through this necessary reduction in bacterial population, to make room for a further multiplication, is accomplished in mass by the removal of excess sludge in the activated sludge process, this is not true of the trickling filter. Here the natural enemies of the bacteria; - the protozoa, other bacteria eating plankton and larvae - must carry the burden of responsibility of keeping the bacterial population reduced, as there is no other means, except for an occasional unloading of the filters. This may occur more frequently in filters if the population of bacterial enemies becomes reduced.

Another factor which must be considered is that in the oxidation process there are three elements; (1) living bacteria, (2) food material and (3) oxygen. If aerobic biological oxidation is to occur, there must be an intimate and continuous admixture of these three. In the activated sludge process this is accomplished by aeration, which adds oxygen and distributes the flocs, with a continuous inflow of fresh food. With the trickling filter this is brought about, frequently less effectively, by a slow distribution of the bacterial food over the entire surface with aeration from up-current of air, the bacterial flocs remaining stationary.

Although it is desired to emphasize the primary importance of bacteria in sewage disposal by biological factors and the fundamentals of this process, it is not desired to create the impression that these zoogleal bacteria are the only ones which can promote oxidation. They are the ones which oxidize certain types of carbonaceous material. Other groups feeding on ammonia and amines produce nitrification, others utilizing nitrites produce nitrafication. Cultures are available which will reduce a standard phenol solution from 500 p.p.m. to 100 p.p.m. of phenol overnight. It is firmly believed that there are present in normal domestic sewage

INDICATOR ORGANISMS OF POLLUTION

(Mr. H. F. Clark)

1. Water and its Relation to Life

Water is the most important single essential for plant and animal life. This compound of hydrogen and oxygen makes up approximately two-thirds the body weight of man. Furthermore, with only one available, man can survive longer on a diet of water and with less discomfort, than he can with only food. Thus a more or less adequate water supply has been demanded by mankind from the dawn of civilization down through the ages.

2. "Satisfactory Water Supply"

The idea of a "satisfactory supply" has always been associated with water. As a descriptive term for water, the meaning has changed with each development in science and engineering. In the beginning clear water with no unpleasant taste was "satisfactory." Today, we place the emphasis on safety.

3. Layman's Judgment of Water Quality

The average consumer judges water on the basis of color, odor, taste and clearness. Of all these, taste is most important, for the layman is apt to associate good taste with safety while unpleasant tastes are considered harmful.

4. Bacteriology and Water Quality

Before the discovery of bacteriology Van Leeuwenhoek had described "minute animalicules," probably protozoa, in waters. These were visible through the newly discovered microscope of the Dutch merchant. Independently Snow and Budd in England had associated the quality of water with the incidence of intestinal disease on statistical evidence alone.

The epoch-making discoveries of Pasteur after 1855 precipitated an intensive investigation in the field of bacteriology. In rapid succession Eberth (1880) isolated the typhoid organism, Koch in 1884 described the cause of cholera and Escherich the following year isolated members of the coli and aerogenes groups.

Numerous studies, discoveries and surveys have followed to add to our knowledge. Over half a century, data was accummulated to develop the separate science of "water bacteriology."

5. Pathogenic Bacteria in Water

In view of the association of water with the spread of intestinal diseases, a natural interest developed in methods for isolation of water-borne pathogens. Methods have been developed for isolation of the typhoid organism, members of the Salmonella and Shigella groups, cholera and others. New research is constently improving these methods. A search of the literature reveals many instances of their isolation and correlation with waterborne epidemics. It should be noted coliform organisms are always associated with the intestinal pathogens in these isolations.

6. Pathogenic Organisms as Indicator Organisms

Isolation of pathogens from water supply is a valuable research tool and a measure in epidemic studies but it has no place in the routine examination of water as an index of pollution.

By the time a pathogenic organism is discovered in the water, the harm has been done. The water was consumed long before the identification of the pathogen is completed. The epidemic has started in the incubation period before the laboratory discovers it. Furthermore, with our present methods the isolation is tedious and laborious and requires skill of the highest order.

It is obvious pathogens will not serve as an index of pollution for the sanitary quality of water. Thus it was necessary to investigate other bacteriological methods.

7. Attributes of Ideal Pollution Test

The essential attributes of the ideal test for pollution should:

A. Yield uniform results with most types of water such as -
  a. Surface
  b. Deep and shallow well
  c. River
  d. Samples during all stages of treatment
  e. Sewage effluents

B. Indicate bacteriological safety of pure water.
C. Indicate approximate degree of pollution of impure water.
D. Require a minimum of time, skill, labor and material in order to have rapid results at small cost.
E. Have sufficient accuracy to satisfy demands of safety in routine procedures.
F. Expandable procedures for special investigations in unusual circumstances.

The ideal test is hidden somewhere in the unexplored unknown. Our rapid progress of the past would indicate improvement will result from new discoveries.

8. Sewage Organisms as Indicators

The practical approach to the ideal test for the bacterial quality of water would be one that "determines directly whether particular organisms characteristic of sewage" and harmless to man "are, or are not, actually present in the water." (1)

Footnote:
(1) Prescott, Winslow and McCrady (1945). Water Bacteriology. page 241

Returning to Escherich's discovery of coli and aerogenes groups in fecal material, these organisms were investigated as a pollution index.

The coliform group became an index of pollution in water bacteriology for this group of organisms, insofar as known, is considered to approach most nearly the definition as one of the "characteristic sewage organism." There is excellent evidence to recommend this group. The coliforms are:

A. Present in the intestinal tract of animals.
B. Constantly eliminated in large numbers in fecal material.
C. Seldom found in non-polluted waters in any quantity.
D. Respond to environmental changes outside the gut in a manner similar to many intestinal pathogens.
E. The group is relatively easy to detect in extremely small numbers.
F. The group does not produce epidemic disease.

Because of these facts and the knowledge that waters containing sewage pollution may equally well carry intestinal pathogens, is the reason the coliform group detection test has assumed importance as a factor in judging sanitary quality of water.

Other organisms have been proposed to supplement, or replace, the coliform group. The Enterococcus group, Streptococcus faecalis, Escherichia coli and members of the spore-forming, lactose-fermenting anaerobes (Clostridium) are current examples.

9. The Coliform Group

As an indicator organism the coliform group includes all aerobic and facultative anaerobic gram-negative, non-sporulating bacteria which ferment lactose with gas formation. This is the definition on page 193 of Standard Methods (9th Ed) with the exception that the word "bacteria" has been substituted for the word "bacilli." This change is in keeping with the modern classification of microorganisms in which the term "Bacilli" is restricted to the aerobic, spore-forming rods.

In the above definition a group of organisms is covered in the specification - not a single species, nor even a single genus. The current edition of Standard Methods permits no modification of this standard such as substituting the Escherichia group.

There is no differentiation into "fecal" and "non-fecal" types. Again quoting Standard Methods, the technologist is cautioned against the "fecal vs non-fecal strain" controversy with the statement that "any attempt to evaluate a drinking water on the basis of a distinction between these two types is regarded as unwarranted." The significance of strain differentiation demands further investigation. A reliable and simple differential method would, without doubt, increase the present restrictive standards for potable water.

As a result of the application of the coliform procedure in the examination of water and its correlation with epidemiological studies of enteric fever incidence, a considerable amount of information has been accummulated in regard to safe and unsafe waters. It is on the foundation of such information that the coliform standards have been based.

10. Deficiencies of Coliform Test

At the same time the shortcomings of the current method have been recognized. Continuous effort has been directed to the development of a better criterion. Such improvements have followed these major lines of investigation:

A. Improve and simplify present methods.
B. Select another indicator organism that would give more information.
C. Utilize one genus of coliform group.

11. Studies to Improve and Simplify Current Method

The most desired improvement of the current method would consist of devising a medium which would stimulate to rapid growth a single organism of the coliform group and inhibit, or suppress, all other varieties of micro-organisms. Although this visionary ideal medium has not been discovered, there has been steady progress toward more selective media for isolation and confirmation. In 1906 a medium containing one per cent glucose was in use. The first major improvement was the substitution of lactose for glucose. This change reduced materially the number of false positive presumptive tests.

Now a major field of investigation is the modification of standard lactose broth by omission of some ingredients and addition of other substances to inhibit the growth of non-coliform organisms.

The observation of Churchman (1912) that some dyes would exhibit selective suppression on certain species or groups of bacteria added impetus to this work. Such dyes as brilliant green, crystal violet, fuchsin and many others have been tried. Other agents, alone or in combination with dyes, such as bile salts and surface tension depressents as sodium lauryl sulfate are used. Perry (1943) has used elevated incubation temperatures as a suppressive measure.

At various times brilliant green lactose bile broth, crystal violet lactose broth, fuchsin lactose broth, formate ricinoleate broth, lauryl sulfate, tryptose broth and McConkey's broth have been purposed as a presumptive medium.

With the exception of lauryl sulfate tryptose broth, the finding that these media, under certain conditions, will inhibit the growth of coliform organisms to a greater or lesser degree, thus rendering them unfit for use as a presumptive medium.

In general the use of an inhibiting substance must be a compromise. Substances such as brilliant green will inhibit Staphylococcus aureus in 1-10 million dilution in broth while Escherichia coli is inhibited only in a 1-20,000 dilution. The addition of bile salts further reduces the toxicity of B. G. for various organisms.

Some of the media which were rejected as presumptive tests had their greatest use as a confirmatory medium. The present brilliant green lactose bile broth as a confirmatory medium is the most important advance for coliform confirmation made in the last fifty years.

A possible improvement in presumptive medium may be the new lauryl sulfate tryptose medium. Sufficient data on comparative studies between this medium and the lactose broth are not available at this time. Correlation between the two medium is good on potable waters but datum with relatively impure waters is lacking.

12. Other Organisms as Pollution Indicators

There is not time to give more than passing mention to the non-coliform organisms which have been suggested as indicators of sewage pollution in water. Any organism that has its normal habitat in the intestinal tract of man can be considered as a candidate for membership in this group.

Streptococcus

A leading "pretender" as an indicator of pollution is the Streptococcus.

The Streptococcus as a group has wide distribution. Many of them are pathogenic for man and animals. Some strains are strict parasites while others are saprophytic in existence. Many are fastidious in their growth requirements while others will grow with ease.

The sewage Streptococcus or Enterococcus have been proposed as an indicator organism on the basis that:

A. They are present in feces and sewage and are found in known polluted waters.

B. They are not found in pure waters, virgin soil and sites out of contact with human and animal life.

C. They do not multiply outside the animal body (except in such media as milk). (Suckling, Exam. Waters & Water Supplies - (1943) Blakiston & Co.)

There is not complete agreement on these three points. Evidence indicates the enterococcus does not multiply in water. Whether they persist longer in water or disappear at about the same rate as coliforms is a disputed point.

The sewage Streptococcus is never present in as large numbers as coliforms. The Streptococcus-coliform ratio varies from 1-100 down to 1-10. As the Streptococcus detection methods are apparently no more sensitive than coliform methods and as the pollution density is less, this would have the practical effect of reducing the sensitivity of the method.

Streptococcus classification methods have attempted to distinguish between human and animal strains. Mannitol-positive strains "do appear more common in human feces and raffinose-positive strains more common in bovine feces" but the separation on this basis has no place in evaluating the sanitary quality of a water supply.

Streptococcus methods offer a promising line of investigation. At this time the Streptococcus index is not a satisfactory substitute for the coliform group. Its greatest value is as a corroborative test where the coliform datum is suspected.

The Anaerobes

Spore-forming, lactose-fermenting anaerobes have been used as indicator organisms in water. The anaerobic organism is usually referred to as Clostridium welchii in England and Clostridium perfringens in America.

The spore-forming Clostridium is unsuitable as indicator organisms for the following reasons. (Levine)

a. Extreme resistance to destruction and long survival.

b. Abundant in animal manure, cultivated soil and decomposing organic material.

c. Failure to correlate results of sanitary survey.

d. Small difference in numbers in heavily polluted and pure waters gives no evidence of the degree of pollution.

The major interest in anaerobes at present centers about the occasional false positive presumptive test and its carry over to brilliant green bile broth tube with gas and decolorization. The anaerobe can be eliminated by its failure to grow in formate ricinoleate broth.

The problem of finding a better indicator organism is one of paramount interest. Some show promise but none, at present, compete with the fifty odd years of experience with the coliform estimation and its correlated data.

Escherichia Coli as an Indicator

Many years ago, Bacterium coli was considered to be a single species. It was sufficient to demonstrate the following characteristics:

1. A rod shaped organism not stained by Gram's method.
2. Aerobic and facultative anaerobic organism.
3. Not able to produce spores, and,
4. Fermentation of lactose with gas production.

A not too recent count reveals at least thirty odd species of organisms will fit the above definition. As new methods and tests were devised, this so-called single species was devided into more and more species. Now this group of organisms is considered under the collective term of "coliform organisms."

Hundreds of research workers have added their contributions to the studies to determine the characteristic of the bacteria included in the coliform group so that the ideal bacterial criterion of pollution might be defined. From this mass of data has evolved a classification of the coliforms into Escherichia coli group, the intermediate group and Aerobacter group. These differences are not sharp, well defined distinctions but are rather differences that shade from one variety to another on the basis of the interpretation of results from multiple testing procedures. That is the problem involved in the use of Escherichia coli as the indicator organism. It is further complicated by the question of what intermediate varieties to include with the Escherichia coli as a pollution indicator organism.

Lewis and Pittman (1) in their work found no significant difference in the ratio of Escherichia coli to coliforms in polluted water and in water of high sanitary quality. Ruchhoft, et. al (2) found the ratio of Escherichia coli to coliforms in a sewage effluent and at the pumping station to be about the same although the coliform M. P. N. count was 13,815 and 0.219 per mililiter, respectively. (2) Taylor (3) found the Escherichia coli ratio of no help

Footnotes:

(1) Jour. Am. W. W. Assoc. (1928):78
(2) Jour. Bact. 25: (1933):143
(3) Jour. Hyg. 41: (1941):17

in estimating the sanitary quality of water in English lakes.

A summation of the evidence of many workers warrants the following conclusions.

1. The ratio of Escherichia coli to that of other coliforms is not an index of water quality.
2. Presence of Escherichia coli is no more significant than other members of the coliform group.
3. The additional labor involved in the identification of Escherichia coli in the presence of other coliforms is not justified on the basis of the information attained.
4. The sanitary quality of water is dependent on the total number of coliform organisms present. Density is an indication of pollution.

SUMMARY

From a bacteriological point of view the coliform group is the best available indicator for use in the estimation of pollution of waters and in the sanitary evaluation of pure waters of potable quality. The coliform test is a quantitative test. Significance in its interpretation is dependent on determining the density (or most probable number). Only when the count exceeds a normally expected minimum number of coliform organisms is pollution indicated. Few samples of water are completely free from coliforms when a sufficiently large sample is examined. The established standard of the U.S.P.H.S. for drinking water recognizes the possible occurrence of an occasional coliform.

In the foregoing discussion it was evident that pathogenic organisms could not be used as indicator organisms. Their unsuitability is based on infection of the public before their discovery, their abnormal occurrence in the feces of a small percent of any group, and their small number in comparison to other intestinal type organisms.

It is freely admitted that there are many types of organisms normally found in the intestinal tract of man not belonging to the "coliform group." Some of these organisms were considered as "indicator organisms." The available data eliminated each one for one or more reasons.

The substitution of Escherichia coli for the present coliform standard was unsuited because it gives no additional information for the increased labor and because there is not sufficient epidemic studies to correlate the Escherichia coli index.

Quoting Butterfield in 1945, he states that "...while it is desirable to seek for better bacterial criteria of pollution in water, and these researches should be continued, the results of such studies to date have not produced a criterion which may be considered to have satisfactorily replaced the coliform group." This summation is equally true today.

There is no place for error in the use of indicators for pollution.

Preparation for Bacteriological Examination and Sample Collection

by

(C. W. Chambers)

General Outline

I. Sampling Equipment

(1) Depth sampler and accessory equipment.
(2) Shallow water sampler.
(3) Torch or substitute.
(4) Sample case.
(5) Thermometers.
(6) Identification tags.
(7) G. S. Bottles of adequate size and design.

II. Preparation of Sampling Equipment - Design of Special Equipment

In the analysis of water and sewage it is essential to have adequate sampling equipment available. In most instances a sterile bottle is the only sampler required when collecting samples from drinking water supplies and swimming pools. You are each being provided with a copy of P.H.S. reprint No. 2263 which includes information on samplers used in specialized work. Equipment of this nature must necessarily be custom built as it is not available through scientific supply houses. Such samplers as are on hand will be demonstrated.

Samples must be transported in cases in which storage will be at temperatures conforming to the specifications set forth in "Standard Methods for the Examination of Water and Sewage, Ninth Edition" (1) hereinafter referred to as "the standard". The standard does not contain any information relative to the design of sample cases. However, adequate design should provide the following:

(1) Ice capacity commensurate with the anticipated transport time.
(2) A means of keeping the bottles in an upright position.
(3) Overflow or other method of preventing contamination of sample bottles by residual ice water.
(4) General convenience when in use.

All equipment should be prepared in accordance with the standard except in instances where it does not cover the items discussed. In case of the latter, preparation should be governed by the use of procedures based on sound fundamental laboratory practice. It is appreciated that all sanitary engineers and associated laboratory personnel make an honest effort to become thoroughly familiar with standard procedures. Accordingly, it is not our intention to repeat in detail everything included in the standard. We shall emphasize some of the more important procedures which, on the basis of laboratory surveys and personal observation, are most frequently neglected. In preparing sample bottles they should:

(1) Be properly cleaned.
(2) Contain 0.02 - 0.05 g. dry $Na_2S_2O_3$ unless absolutely sure that chlorine will not be present in the samples collected. The question of including $Na_2S_2O_3$ in sample bottles has been subject to much abuse. Even a very low concentration of chlorine (.04 ppm at pH .70), if it exists as free chlorine, can kill all E. coli present in one minute. With this in mind it should not be necessary to point out the possibilities; especially if any consideration is allowed for the transport times and temperatures often used. Those who desire more complete information regarding the effect of chlorine and chloramine are referred to P.H.S. reprint Nos. 2530, 2593 and 2693.
(3) Have neck and stopper shielded with metal foil or suitable paper substitute.
(4) Be sterilized at either 170°C in dry heat for one hour or for 30 minutes steam pressure at 15 pounds and 121°C.
(5) Be packaged in such a manner as will eliminate contamination subject to normal handling.

III. Preparation of Media and Other Equipment.

A. All glassware and apparatus must be thoroughly cleaned and sterilized for one hour at 170°C dry heat. In all sterilization processes loading of the sterilizer should be such as will insure that all articles processed will be heated for the time, and to the temperature, recommended in the standard. All media shall be prepared according to the recommendations of the standard.

B. Your attention is directed to some particularly critical considerations: lactose broth and lauryl sulphate tryptose broth should always be made up and tubed in such a concentration and in such volume that, after receiving the appropriate amount of sample, the final concentration of ingredients will equal that indicated for these media. These media and gelatin media too, should be sterilized at 15 pounds pressure for 15 minutes, but the total time they are exposed to heat should not exceed 30 minutes. Furthermore, immediately after sterilization they should be cooled rapidly. When a complex sugar such as lactose is heated to a high temperature for a considerable period of time it hydrolyzes to simpler forms, in the case of lactose, to dextrose and galactose. All species of the genus Proteus produce gas in media containing dextrose or galactose though none of them ever produce gas in media containing pure lactose, thus it is obvious that any breakdown of lactose during sterilization could result in serious errors.

C. Other errors common in the preparation of media are:

(1) Failure to check the pH and, if necessary, make adjustments.

(2) Failure to replace water loss due to evaporation.

D. Dilution Water.

(1) Use either the dilution water as mentioned under reagents in the standard on page 140, item 2.1, or tap water. As tap waters vary in different locations it is suggested that phosphate water be used. This is especially desirable when viewed from the state level. In any given state there may be many different laboratories making bacteriological analsyses of water. In each laboratory if tap water were used for dilution purposes, the results would be

subject to the peculiarities of that particular tap water. Consequently, for purposes of comparison, it is distinctly advantageous to the state engineer to have all results based on analyses in which the methods are comparable. Furthermore, results may not even be comparable in any one laboratory due to seasonal variations in the tap water when used for dilution purposes whereas, by using synthetic dilution water throughout the year the effects of seasonal variations on the method proper are negligible. Distilled water or physiological saline solution should never be used as a substitute. Waters from six different sources to which bacteria were added, using the natural water in each experiment as the source of bacteria, resulted in the bacterial colony counts shown in the following table.

*SLIDE I.

The volume of water in dilution blanks, after sterilization, should never vary more than two percent from that recommended in the standard. Periodic pH and toxicity determinations on the sterilized dilution water are desirable.

IV. Sample Collection:

The two prime objectives in collecting a sample for bacteriological examination are:

(1) It must be taken in such a manner as to be truly representative of conditions prevailing at the time the sample is collected.

(2) The bacterial numbers must be maintained without any significant changes until the sample is planted. To base recommendations on laboratory results obtained from samples which do not substantially fulfill those requirements indicates (1) negligence or (2) ignorance of the dangers inherent in

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*Table from P.H.S. reprint No. 1580

such practice.

Sampling data should be complete and should include time, temperature, location and any special pertinent information.

A. Potable Water:

(1) In taking a sample from a tap the faucet should be flamed and the water allowed to flow at full-stream for at least 5 minutes. If the tap is not in relatively constant use at least 15 minutes flow time should be allowed. In the act of collecting a sample the valve should not be changed from the position it was in while the 5 minute flow was in progress. In taking samples from a pump flame the spout and operate the pump at least 10 minutes or longer where possible.

No figures are available on the counts obtained immediately after pumping started. In water from a well little used during the 36 hour period preceding sampling the first water drawn had a total count of 5,000 per ml while after one half hour of pumping, the count was reduced to 35 per ml.

B. Stream Sampling

It is important that a means be provided whereby the sampling station may be accurately re-located on successive trips. This is particularly difficult in wide streams where it is necessary to resort to the use of boats and quarter-point sampling. Special data such as variations in flow and shifts in the flow from sewer outfalls should be noted. Being a good observer is an essential attribute of a good sample collector.

C. Bathing Beaches and Swimming Pools

Because there may be a high concentration of Cl_2 in such waters it is particularly important that no $Na_2S_2O_3$ be lost from the bottles in the course of sampling.

D. Ice Samples

In sampling ice representative solid blocks should be split with a sterile chisel. Using a sterile ice pick, chip pieces from the freshly exposed surface allowing them to fall into a sterile bottle. If the ice is melting care should be exercised to prevent flow from contaminated surfaces from reaching the sample area. Crushed ice should be sampled during delivery in the customer's establishment.

E. Sewage and Sea Water Samples.

The general precautions previously mentioned apply these types of samples also. Sewage and sea water samples are particularly susceptible to rapid change if transportation and storage time and temperature are not carefully controlled.

Standard Methods for the Examination of Water and Sewage (1) on page 191, Section IV B. Storage and Transportation, in part reads as follows: "Because of the rapid and often extensive changes which may take place in the bacterial flora of bottled samples when stored even at temperatures as low as 10°C., it is urged, as a matter of importance, that all samples be examined as promptly as possible after collection. THE TIME ALLOWED FOR STORAGE OR TRANSPORTATION OF A BACTERIAL SAMPLE BETWEEN THE FILLING OF THE SAMPLE BOTTLE AND THE BEGINNING OF THE ANALYSIS SHOULD NOT BE MORE THAN SIX HOURS FOR IMPURE WATERS AND NOT MORE THAN TWELVE HOURS FOR RELATIVELY PURE WATERS. DURING THE PERIOD OF STORAGE, THE TEMPERATURE SHALL BE KEPT BETWEEN 6°C AND 10°C." This has been a requirement of Standard Methods, in some form in all editions, for about thirty years. Anticipating that this requirement might not be met by all, Standard Methods provides also that, "ANY DEVIATION FROM THE ABOVE LIMITS SHALL BE SO STATED IN MAKING REPORTS," IN ORDER THAT A JUDGMENT MAY BE FORMED AS TO THE RELIABILITY, OR LACK OF RELIABILITY, OF THE RESULTS.

The Drinking Water Standards (2) of the U. S. Public Health Service also subscribe to this requirement for they state, page 7, footnote 2. "This reference (1) shall apply to all details of technique in the bacteriological examination, including the selection and preparation of apparatus and media the collection and handling of samples, and the intervals and conditions of storage allowable between collection and examination of the water sample."

Prescott Winslow and McCrady 1946 (3) make the following statement: "There is evidence, during the past decade, of a tendency to ignore the necessity of icing water samples that are to be examined only for the presence of coliform organisms, even to the extent of sending uniced samples long distances by mail. This tendency is to be deprecated; errors resulting from neglect of icing are almost sure to be on the WRONG side of safety and to lead eventually to pronouncing as safe WATERS THAT ARE FRANKLY DANGEROUS. 'It should be emphasized that the practice of examining uniced samples derives sanction from EXPEDIENCY and does not conform to the conditions best for analysis, ' (4) and the dictum that 'an unchilled sample cannot be accepted as a substitute for a fresh or chilled sample ' (5) reflect the considered opinion of the great majority of experienced water bacteriologists both in the Americas and abroad."

This requirement, namely, the maintenance of samples at a temperature between 6° and 10°C., makes it necessary to ice samples during the interval between collection and examination for at least a portion of each year in the majority of climates. Though extensive changes should be expected in dealing with organisms whose generation time may be as short as 10 to 20 minutes, (and such changes would be expected to vary with temperature), a very considerable opposition to the icing of samples has always existed. This opposition, no doubt, is born of a lack of knowledge of the evidence and also the annoyance and cost which is incident to the icing of samples. A review of the literature which lays the foundation for this requirement should dispel any hesitancy with regard to insisting that it be strictly adhered to.

Early investigators of this problem Jordan and Irons (6) Hale and Media (7) Albert, Hinman and Jordan (8) Rector and Daube (9) Eijken (10) Boruff and Buswell (11) Huss (12) and Berry (13) obtained results which were contradictory in some instances and frequently inconsistent. This evidence indicated definitely that there were factors other than temperature, or in conjunction with it, which influenced the changes in bacterial numbers. "When a bacterium cannot multiply, the only vital activity which can take place is a katabolic wasting away, which soon proves destructive and the higher the temperature the more rapidly the fatal result is reached". (3)

Some of these factors which MAY be responsible for variation in bacterial numbers are:

(1) Toxic matter
(2) Bacteriophage (14)
(3) Auto-agglutination
(4) Electrophoresis
(5) Sunlight
(6) Oxygen tension
(7) Turbidity - Especially R_2O_3
(8) Eutomostraea - Daphne, (15)

(9) Acidity or caustic alkalinity
(10) Presence of food material
(11) Protozoa (16)

Temperature and time of exposure do have an effect. The longer a sample is exposed to the action of an agent; regardless of whether or not it is chemical, biological or otherwise, and so long as it is within the temperature range encountered in nature; the greater will be the effect.

In 1914 the Public Health Service at its Cincinnati Laboratory initiated studies on the bacterial changes occuring in water samples. These studies were continued for about ten years. Items considered in this investigation included, (1) the time and temperature of storage, (2) The size and material of the sample container, (materials used were glass, wood, metal, paraffin, concrete and collodion sacs), (3) effect of light and air surface exposure, and (4) influence of alterations in the sample, (centrifuging, filtrating, adding dilution or pollution). The results of these studies have been reported in part by Butterfield (17) (18) and by Hoskins and Butterfield (19).

IN GENERAL, these reports indicated that regardless of the temperature of storage, THE BACTERIA IN SAMPLES HELD AFTER COLLECTION INCREASED TO A MAXIMUM AND THEN DECREASED. However, this was not always true, occasional samples were found in which the bacteria present decreased in mumber continuously from the time of collection. This was particularly true in samples of RELATIVELY PURE WATER and in samples of water containing substances TOXIC to bacteria. The rate of increase or the time required to reach the maximum, varied with the temperature of storage, the rate being most rapid at the higher temperature. To reach the maximum required an average of about 8 to 12 hours at 37° C., 22 to 24 hours at 20°C., 50 hours at 10°C., and 13 days * (20) at 2°C. There was a considerable variation in these time periods with samples from various sources and a very wide divergence in the magnitude of the increase observed, from 121 to 39,000 per cent. The nature of the vessel holding the sample, the presence or absence of an air surface above the sample, provision for or omission of, agitation, or exposure to moderate light, apparently did not alter the trends of the bacterial changes observed. On the other hand filtration, centrifuging, adding dilution or pollution, tended to increase markedly the changes occurring in bacterial numbers.

SLIDES I, II, AND III

Ellison et al (21) as a result of their studies do not favor the icing of samples. They insist that the correct result can be obtained more easily by planting the sample very soon after collection. This is an ideal which cannot always be realized. In their case, water plant control, it was achievable. This procedure may be followed without icing but $Na_2S_2O_3$ should still be used. (22) Kelso (23) found that 50 PER CENT OF COLIFORM TESTS OF UNICED SAMPLES WERE UNRELIABLE AND 90 PER CENT OF THE COLIFORM TESTS ON ICED SAMPLES WERE RELIABLE. His tests were on shipped samples with maximum time of 48 hours.

* Data for this temperature were reported by Greenfield, et al (14)

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1. Journal of bacteriology 1928 Vol. 16 page 260 table 2
2. " " " " " " " 261 Chart 2
3. Sewage Work Journal 1933 " 5 " 612 Figure 5

Damm (24) found that icing proved to be a satisfactory procedure. However, he states that IF the water is of a degree of purity such that THE PLATE COUNT AT THE TIME OF COLLECTION IS LESS THAN ONE PER ML THE SAMPLES MAY BE HELD SAFELY WITHOUT ICING; OTHERWISE ALL SAMPLES MUST BE KEPT COLD.

Caldwell and Parr (25) summarize the results of their study as follows: "Comparison of RECOVERY OF COLON GROUP BACTERIA FROM iced and uniced SAMPLES EXAMINED AT VARYING PERIODS INDICATES A RAPID DEATH RATE OF B. COLI in bottled samples of contaminated waters, WHICH IS PROGRESSIBELY GREATER AS THE PERIOD OF HOLDING INCREASES. ICING, though of secondary importance, OFFERS A DISTINCT ADVANTAGE. Direct inoculations were found practicable and showed marked superiority over any other method." By direct inoculation the authors mean that the bacteriologist collects the sample and plants it immediately as collected.

Tanner and Schneider (26) from the results of their study on the effect of the temperature of storage on the bacteria in water samples conclude in part as follows: "The number of viable organisms present in water after storage in the refrigerator (0° to 7° C.) varied but slightly from the original. The ability to ferment lactose is not inhibited by low temperatures of storage but seemed to be maintained over long periods in iced specimens. THE DATA COLLECTED IN THIS INVESTIGATION INDICATE IN PRACTICALLY ALL CASES THAT WATER SAMPLES SHOULD BE KEPT COLD BETWEEN COLLECTION AND ANALYSIS. This would indicate that icing of samples as provided in Standard Methods for the examination of water and sewage is desirable."

Webster and Raghavachari (5) in Madras have investigated the bacteriological examination of waters at the source and after transport to a distant laboratory. They examined 104 sets of samples, by planting at the source as collected and after shipping to the laboratory, iced and uniced. In 75 per cent of the samples coliforms were fewer in the uniced portions. In 19 pairs of samples the test for true coli was positive in the iced, negative in the uniced portions. THEY DECIDED THAT when planting at the immediate time of collection is not practicable, THE TROUBLE AND EXPENSE OF ICING IS ENTIRELY JUSTIFIED AND THAT UNICED SAMPLES CANNOT BE ACCEPTED AS A SUBSTITUTE FOR FRESH OR CHILLED SAMPLES.

Keiper and Flinn (27) from their studies on the influence of time and temperature of storage on the results obtained in the bacteriological analysis of water samples conclude that, "WATER SAMPLES subjected to the same conditions as samples sent through the mail, i.e. , STORED AT ROOM TEMPERATURE FOR 24 HOURS and 48 hours AFTER COLLECTION, showed increased plate counts and increases in Esch. coli. Such samples DO NOT YIELD RELIABLE DATA FOR JUDGING THE CHARACTER OF A WATER SUPPLY."

Harvey (28) investigating the effects of storage of sea water in glass containers found that there was a rapid and extensive increase in bacterial numbers. He believes that the increase is caused by a concentration of the food material in the water on the glass surfaces.

Undoubtedly this brief review has not included all of the articles in the literature on this subject. However, it is believed that a fair cross section of the available evidence has been presented. While there is a wide variation in the ideas concerning the causes of bacterial changes in stored samples there is a marked unanimity of opinion as to the beneficial effect of maintaining samples at a low temperature during the interval between collection and examination. With this background of evidence it would appear that the requirement of Standard Methods for the Examination of Water andSewage for stored samples that, "During the period of storage, the temperature shall be kept between 6°C. and 10° C.", is not only entirely justified, it is also quite essential IF RELIABLE RESULTS ARE TO BE OBTAINED. It has been our policy to observe this requirement consistently if the period of holding of the water sample, between the time of collection and examination, is more than 20 minutes.

In closing it is requested that you consider the following questions:

Would you sanction the continued operation of a water works in such a manner that 20% of the samples from the distribution system contained coliform organisms? In your state laboratory, would you tolerate a water bacteriologist who insisted on using non-standard methods for the determination of coliform organisms and as a result reported as negative for E. Coli, 20% of the water samples which would have been positive if he had used the approved method? If not, why then consider as satisfactory, analyses on samples which, due to improper shipping, may readily result in even greater errors?

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Relative Value of Media for the Detection of the Coliform Group in Water
(Mr. Chambers)

1. Presumptive Media (1, 4, 6, 7, 8, 12, 13, 14)

   A. Lactose Broth

      (1) Still the most productive proven medium.
      (2) Sporulating gas formers not inhibited.
      (3) Subject to hydrolysis.

   B. Possibilities of Newer Media - L. S. T. and E. C.

      (1) Should yield less positive presumptive tubes than lactose and as many or more positive confirmed or completed tests on the same samples.

      (2) On comparative research at this station two types of water were used.

         (a) Relatively pure waters.
         (b) Grossly polluted waters.

      (3) In examination of a sample for each type of media under (2, a and b above):

         (a) Three or more concentrations were planted in decimal dilution.
         (b) Three or 5 identical portions at each dilution of each sample were planted.
         (c) Triplicate sets of presumptive tubes were planted on E. C.
         (d) Each type of media incubated at 37°C with E. C. incubated in addition, at 44.0°C and 45.5°C in air and in a water bath.
         (e) All samples were subjected to the completed test. All samples positive with the completed test were tested for indole, MR - V.P. and ability to grow on citrate medium.

      (4) Comparison of results obtained.

         (a) Comparisons were made on determinant samples only, which means that one or more tubes had to be positive in all five of the different types of presumptive tests-tables 2 and 3. (1)

II. Confirmatory Media (2, 3, 4, 8, 9, 10, 11, 13, 14)

A. Brilliant Green Lactose Bile Broth 2%

(1) Eliminates most spore formers.

(2) Coliforms do well.

(a) Slightly higher M.P.N. obtained using B.G.B. than by the completed test, using a wide variety of waters.

(b) May substitute confirmed tests for completed test if B.G.B. broth 2% is used but never when using other media.

(c) What of suggestion that B.G.B. 24 hour reading be substituted for completed test? Only a minor difference between completed and B.G.B. at 48 hours, with slight added safety factor using B.G.B. However, at 24 hours the B.G.B. result could be badly in error, on the wrong side of safety, and increasingly so as the degree of treatment to which the water has been subjected increases. Figure 2 (2).

B. Crystal Violet Broth

(1) Permissible alternate where completed tests indicate a maximum yield of coliforms. Why not just use B.G.B. and eliminate the necessity for parallel tests?

C. Endo or E.M.B.

(1) Most effective if transfer is made immediately after gas production in presumptive medium is first detected, progressively less effective as transfer time is delayed.

(2) The personal variation in reading rules these media out for use where final conclusions on finished waters are to be based on the confirmed test. In picking colonies for the completed test, on the basis of mathematical probability and personal factor combined, it is advisable to pick a considerable number of colonies from each plate.

III. Media for the Completed Test (4, 5, 8, 13, 14)

A. Formate Ricinoleate Broth

(1) Very inhibititive to lactose fermenting spore formers.

(2) Not very productive of coliforms. In important decisions, where a completed test does not provide a clearly positive or negative result, resort to the most complete identification of the organism possible.

IV. Solid Media Direct Count vs. Present Standard Procedure (15, 16)

A. The following weaknesses are common, in a considerable degree, to all the media in this classification:

(1) Superimposed effect of inhibitors used.

(2) Personal factor - complete exclusion, or even reasonably good correlation of results between different workers, is impossible with any medium the use of which is predicated on personal interpretation of the degree of change of color where absolute "color standards" cannot be prepared and used in this final reading.

(3) Oxidation reduction potentials, pH, and light all affect the indicators incorporated in such media.

(4) The large Petri dishes and amount of media necessary to plant 5 ml and 10 ml quantities, to say nothing of 100 ml quantities of samples.

(5) Borderline colonies may constitute over 90% of total on some samples.

(6) Incubation time critical.

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TECHNIQUE OF THE BACTERIOLOGICAL EXAMINATION OF WATER

(H. F. Clark)

This discussion of the bacteriological examination of water is based upon the technical methods described on pages 190 to 208, "STANDARD METHODS FOR THE EXAMINATION OF WATER AND SEWAGE (9th Ed.) 1946", published by the American Public Health Association. Following exactly these procedures described in Standard Methods will give equal value to the data obtained from any laboratory because such routine will eliminate small individual differences due to variation in methods. Such a policy also protects the administrator of the laboratory from criticism of the methods he has adopted as there is a uniform use throughout the nation. Newer methods resulting from research are included in Standard Methods as rapidly as they are proved reliable through investigation and evaluation, thus enabling the laboratory administration to keep reasonably up to date in its operation.

The routine bacteriological examination of water consists of a plate count and an estimation of the coliform group density in the sample. The coliform density however, is of far greater importance in the sanitary evaluation of water. Too much emphasis cannot be laid on the observations and procedures outlined in Standard Methods for the performance of these tests. This discussion might better be entitled "Appreciation and Limitations of Bacterial Procedures."

Collection, transportation and storage of samples have been described in detail by another speaker. With an apology for repetition, it is most important that the time between collection and the initiation of the bacterial examination be reduced to the minimum. Furthermore, the temperature of 10°C. or lower must be maintained during the transportation and storage of the samples. Sterile glassware, media and other equipment have also been discussed in previous periods, but there will be some repetition.

The water used in making dilutions must, after sterilization, have:

1. No bactericidal nor bacteristatic properties to the usual organisms found in water when exposed to them for a reasonable length of time (about 30 minutes);
2. Freedom from particles or precipitate which might be confused with bacterial colonies during counting;
3. Maintenance of a hydrogen ion concentration within biological growth limits, and;
4. Sufficient mineral elements to prevent excessive changes in osmotic pressure (osmotic shock) on the addition of living organisms.

Phosphate dilution water fulfills all of these requirements. It is prepared by adding 1.25 mls. of phosphate stock buffer solution to a liter of distilled water. After mixing measured quantities are sterilized by autoclaving. The stock buffer solution consists of 34 grams of potassium dehydrogen phosphate dissolved in 500 mls. of distilled water, adjusted to a pH of 7.2 with normal sodium hydroxide solution and diluting the mixture to 1 liter. Formula C water may be used as a substitute for the phosphate dilution water although it should be kept in mind that some precipitate is formed during its sterilization. The suitability of tap water in your locality is an individual problem as it may or may not conform to the specifications outlined for a satisfactory dilution water. Some laboratories use distilled water alone or physiological salt solution. This practice should never be permitted, for each of these liquids may exert toxic action with a resulting reduction in the plate count.

The accuracy of the counts are dependent on the accuracy of all the individual steps in making the count. The sterile blanks must be accurately measured and all equipment such as pipettes, incubators, etc. which function quantitatively or measure will have to perform within the limits specified in Standard Methods. If accurate results are to be obtained it must be remembered that operational skill can never compensate for improper or shoddy equipment.

THE PLATE COUNT

The plate count is a method for the enumeration of the number of viable bacteria in the water sample. The results of the plate count are based on the assumptions that:

1. All of the bacteria are viable.
2. Grow well on the medium used.
3. During a short incubation time.
4. With all the mixed strains having the same growth temperature range during incubation, and
5. All organisms are present as individuals (no clumping).

Such an ideal condition never exists in a water sample which may contain many strains of organisms that will require special conditions for their growth. It is not possible to devise a single test which will permit the counting of all viable bacteria in a sample of raw water containing a mixed bacterial population. However, this criticism of plate counts does not deny, nor even minimize their "importance in routine examinations made in connection with the control of water-purification processes." In routine practice any unaccountable shift in the plate count should be investigated immediately.

Nutrient agar and nutrient gelatin are the types of standard media normally used in the making of plate counts. The substitution of tryptone glucose extract agar for the nutrient agar is permitted. This is a common practice in laboratories where the milk and water counts are made by the same personnel for such procedure reduces the possibility of media confusion. Plate counts made with tryptone glucose extract agar medium give about the same values as nutrient agar with water samples and may give larger, more easily counted colonies. All media must be free from precipitate, constant in composition and of proper pH. Dehydrated media of reputable manufacture may be used.

Plates containing more than thirty and less than three hundred colonies are satisfactory for counting. Excessive numbers of colonies on the plate result in low counts due to the suppression of growth caused by crowding (antibiosis). In water where the expected count will exceed three hundred per ml., it becomes necessary to make dilutions of the sample with sterile diluting water in order that the portion planted will fall within these limits as to bacterial population. In the case of a polluted sample of unknown plate count, it becomes necessary to plant a wide range of decimal portions of the water. Portions of the water are always planted in duplicate plates, and in cases where it will not work an undue hardship, the planting in triplicate is recommended.

Before taking samples for planting or making dilutions the container must be shaken. This shaking should be rapid and vigorous with an arc of not less than ten inches. A shaking period of not less than twenty-five times is the minimum. The portion is removed with a clean sterile pipette immediately after shaking. The pipette tip should not descent more than one-half inch below the surface of the liquid when removing a portion, for considerable material may adhere to the outside wall. On the other hand, when making delivery of a sample from a pipette the tip should be brought in contact with the surface of the liquid without touching the neck of the bottle on entering or withdrawing.

The choice of the pipette will depend on the volume to be measured. The delivery type is recommended. For measuring samples from 0.01 to 0.20 ml. the pipette should be a 0.2 ml. capacity graduated in hundredths; for 0.1 to 1.0 ml a one ml. graduated in tenths is satisfactory; and for larger samples up to ten mls. a ten ml. pipette graduated in mls. is used.

The sample (or dilution) is planted in duplicate in sterile Petri dishes. The nutrient agar which has been melted and cooled to approximately 42°C. should be at hand. About ten mls of the melted agar is poured on the sample planted in the dish and mixed thoroughly. During the mixing procedure none of the melted agar should be slopped over the side or on the inside cover of the dish. Mixing is much easier in the dishes with fifteen millimeter depth than in the ten millimeter variety.

The elapsed time for the making of the test is important. Excessive loss of time at this point voids the precautions used in the collection and storage of the samples. Not more than five minutes is used in making the dilutions and the remainder of the test should be completed in another fifteen minutes. The total time required must not exceed twenty minutes. This time factor limits the number of samples which can be removed from the ice box at any one time.

Samples of more than one ml. quantity are not satisfactory in the standard Petri dish. If less than 0.1 ml. of sample is used it becomes awkward to measure, difficult to mix and dries out rapidly in the dish before the addition of the medium. Once any drying occurs in the dish before the media is added, it becomes necessary to discard the plates.

INCUBATION

The plates should be incubated in a dark well-ventilated incubator. The atmosphere must be practically saturated with water vapor (saturated humidity). The incubation temperature depends on the media. Nutrient gelatin plates are always incubated at 20°C (19-21°) for forty-eight hours. Nutrient agar plates may be incubated as for the gelatin ih thé case of a twenty degree count or at 37°C for forty-eight hours. The agar plates are inverted to prevent the water of condensation from forming on the inside surface of the glass. Plate containing gelatin or plates with the tile covers are not inverted during the incubation period.

The results of the plate count must include the time and the temperature of the incubation. Any unusual circumstances are also included in the report.

COUNTING PLATES

Plates with excessive number of colonies give low counts due to supression of growth, overlaping colonies and other factors. The following general principles aid in the counting of plates:

1. Count plates with between 30 and 300 colonies.
2. Use controlled illumination.
3. Use magnification of 1½ diameters.
4. Record only two significant figures per ml., and,
5. Label counts as "standard gelatin plate count," "20° standard agar plate count," "37° standard agar plate count," etc.

The use of the Quebeck Colony Counter or an instrument of similar specification is recommended.

The plate count is calculated in terms of the number of colonies per ml. of original sample. In making such calculations, always include duplicate plates of the same dilution or exclude both plates except when one of the duplicates is obviously in error. In cases where more than one set of duplicate plates were made, equal authority shall be given all sets which fall within the prescribed colony counting range.

COLIFORM GROUP TESTS

DEFINITION - The COLIFORM GROUP includes all aerobic and facultative anaerobic Gram-negative, non-sporulating rods which ferment lactose with gas production. This is the equivalent of the "Bacillus coli group" of Standard Methods prior to the sixth edition and the "coli-aerogenes group" of the subsequent editions.

Identification of the coliform group cannot be accomplished by any single test. However, it can be demonstrated with certainty by the application of a series of elimination or screening procedures in which confusing organisms are dropped along the way.

The estimation of the coliform group density in a water sample is best approached by a decimal dilution method. This is accomplished by calculating the density from data obtained by planting of multiple portions.

Standard procedures for the presence of members of the coliform group have been divided into the "presumptive," "confirmed" and "completed" tests. The number of tests to be applied in any individual sample depends on the purpose in view. Good routine practice makes it necessary to prove beyond doubt that organisms belong to the coliform group by a sufficient number of tests.

PRESUMPTIVE TEST

The consideration of first importance in the presumptive test is the amount and number of samples to be planted. Usually five tubes of each dilution are planted, using three different dilutions which decrease by ten fold each. In pure waters where coliforms are not expected the planting of five 100 ml., one 10 ml. and one 1 ml. portions is often satisfactory. If the coliform density cannot be estimated, it is necessary to plant a wide range of dilutions with at least five tubes for each dilution. The density should not be calculated unless one series of dilutions in the test gives negative data.

The planting of samples of one ml. or larger is accomplished by measuring the specimen into lactose broth fermentation tubes with the customary sterile technique. In no instance, however, must the dilution of the broth by the added sample be such that the active ingredients of the broth are reduced to less than standard strength (beef extract 0.3%, peptone 0.5%, lactose 0.5%). Serial dilutions are made for the planting of less than 0.1 ml. One ml. or 0.1 ml. of such dilutions are planted.

Lactose broth fermentation tubes are incubated at 37°C for forty-eight hours or until gas is produced in the tube, depending on which occurs first.

All lactose broth fermentation tubes are examined at the end of twenty-four hours with the following purpose in mind:

1. Gas produced in inverted vial: positive presumptive test (apply procedure for confirmed test at once).
2. No gas; return to incubator.

After the second twenty-four incubation period, the tubes are again examined. In tubes with the inverted tubes containing:

1. No gas: no coliform organisms present (discontinue test).
2. Gas: doubtful presumptive test (confirm as above).

The presumptive test is a preliminary screening procedure. The absence of any gas formation in the lactose broth fermentation tubes rules out the presence of members of the coliform group in the portion of sample planted. On the other hand any gas formation with forty-eight hours demonstrates the possibility of the presence of coliform organisms.

CONFIRMED TEST

All lactose tubes showing gas at the end of twenty-four hours and any remaining tubes of the series with gas at the end of forty-eight hours should be submitted to the confirmed test. Furthermore the procedure should be started as soon as the gas is discovered in the tubes.

If five tubes each for three decimal dilutions have been used in the presumptive test, it is permissible to confirm only the one or two highest dilutions (smallest portions) of the lactose tubes showing gas. In this case, however, any tubes containing gas which are not submitted to the confirmed test are recorded as containing coliform organisms and the failure to confirm the coliform organisms in the higher dilutions does not change the positive report on the unconfirmed tubes.

At this point the Standard Methods offers alternate procedures using isolation on solid differential media or gas production in liquid differential medium with the latter being the choice of your speaker.

In performing the confirmed test in liquid medium, a loop of broth from the lactose tube showing gas is transferred to a fermentation tube containing brilliant-green lactose bile broth. Shake gently or mix the contents of the lactose tube before taking the transfer from it. Always use a loop at least three mm. in diameter containing between 1/40 ml. and 1/20 ml. Incubate the brilliant-green lactose bile broth tube for forty-eight hours at 37°C. The production of gas in any amount in the fermentation tube within forty-eight hours or less constitutes a CONFIRMED TEST for the coliform group. Absence of gas in the fermentation tube proves the absence of coliform group. If gas with decolorization of brilliant-green lactose broth occurs, the culture should be submitted to the completed test.

The gas containing lactose tube may be confirmed by colony isolation on Eosin Methylene blue or Endo media. Streak one or more plates in such a manner that isolated colonies are obtained on the plate. An isolated colony is one that is at least 0.5 cm. from the nearest colony. The plates are incubated at 37°C for twenty-four hours. The development of typical coliform colonies on the differential medium may be considered a positive confirmed test. The negative results are not easy as many coliform organisms fail to form typical colonies on Endo or methylene-blue medium. Then it becomes necessary to apply the "completed test" to atypical colonies. The problem is further complicated by the personal evaluation of the work as to exactly which colonies and how many are to be identified.

The differentiation of Escherichia and Aerobacter on Eosin Methylene Blue Agar has been described by Levine (Iowa State College of Agric. & Mech. Arts Off. Pub. Bull. 62:20: No. 31, 1921). in the following table:

Characteristic	Escherichia	Aerobaeter
Size	Well-isolated colonies are 2-3 mm. in diameter	Well-isolated colonies are larger, usually 4-6 mm, or more in diameter
Elevation	Colonies slightly raised, surface flat or slightly concave, rarely convex	Colonies considerably raised and markedly convex; occasionally center drops.
Confluence	Neighboring colonies show little tendency to run together	Neighboring colonies run together quickly.
Appearancy by transmitted light	Dark, almost black centers which extend more than 3/4 across diameter of colony; internal structure of central dark portion difficult to discern	Centers deep borwn, not so dark as Escherichia and smaller in proportion to the rest of colony. Internal structure often striated in young colonies.
Appearance by reflected light	Colonies dark, button-like, often concentrically ringed with a greenish metallic sheen	Much lighter than Escherichia. Metallic sheen not observed except rarely in depressed center.

Completed Test

Select a representative number of typical colonies for examination. If no typical colonies are present on the plates, take for consideration the most likely atypical ones. Transfer an inoculum from each colony to a lactose broth fermentation tube and an agar slant. The demonstration of gram-negative, non-spore-forming rods after twenty-four hours incubation and the production of gas within forty-eight hours incubation in the lactose broth fermentation tube constitutes a positive test.

Absence of gas in the lactose broth fermentation tube after forty-eight hours incubation is a negative test.

A combination of gas in the lactose broth tube with spore-bearing rods requires further investigation. Transfer the spore-bearing rod to formate ricinoleate broth. Absence of gas after forty-eight hours incubation indicates only spore-forming lactose fermenters were present.

On the other hand, gas production in the formate ricinoleate broth is an indication of the coliform group.

Estimation of Coliform Group Density

Coliform density is computed and recorded in terms of the "Most Probable Number." The Most Probable Number, commonly called "M.P.N," may not correspond to the density actually present in a given sample, but in the long run it (the MPN) will represent more closely than any other series of numbers the density of gas-producing organisms in the sample examined.(1) Relative accuracy depends chiefly on the number of samples tested for each dilution and the choice of the ideal dilution for any given density of coliform organisms. In the estimation of coliform density:

1. One tube of one dilution has practically no significance
2. One tube each of three dilutions has little more value.
3. Several tubes each of a single dilution may have value only when used with proper coliform density (approximately 50% positive).
4. Several tubes each of two dilutions offers a better chance of securing the proper dilution and yields more information regarding bacterial density.
5. Best practical results are obtained with 5 tubes of each dilution and use of three dilutions with the middle dilution containing both positive and negative tubes.
6. With water of drinking quality a combination of five 100 mil, one ten mil and one - one ml. will give satisfactory result for the Public Health Service Drinking Water Standard.

Only three different dilutions, with each dilution decreasing by ten times, are significant in computing the M.P.N. for coliform density. If more than three dilutions have been tested, choose a dilution at which no smaller results occur and the two following dilutions compose the three usable dilutions. The coliform density is computed by finding the proper combinations of positive and negative tubes in a suitable table such as found on pages 204-5 of Standard Methods.

The Bioassay of Toxic Wastes, in Relation to Industrial Waste Disposal Standards.

(Peter Doudoroff)

A. A bioassay is a quantitative test of the active strength or potency of any physiologically active substance (e.g., a toxic substance, a hormone or vitamin preparation, etc.), involving the use of living biological material as a reagent.

B. Reasons why routine toxicity bioassays are needed in the proper control of industrial waste disposal:

1. Toxicity to aquatic life is the principal objectionable feature of many industrial wastes discharged into streams, so that the usual, standard chemical and bacteriolgoical tests for the measurement of pollution and of the pollutional strength of effluents may be quite inadequate or inapplicable when such industrial wastes are involved.

2. A great variety of toxic components may occur in various industrial wastes, and the concentrations of many of these cannot be determined by chemical means readily enough for routine disposal-control purposes.

3. The nature and the specific toxicity of many of these toxic components of various wastes have not yet been adequately investigated; and their toxicity even to a single organism or "bioreagent" under all possible conditions cannot be definitely ascertained, since it varies greatly with the quality of the diluting water, the nature of other waste components present, and other related factors, because of various complex chemical and physiological interactions.

C. Reasons why waste-disposal regulations or standards expressed in terms of bioassay results are desirable:

1. Laws and regulations pertaining to stream pollution with toxic wastes which are now in effect in most States are too arbitrary or too vague and ambiguous to be effectively and equitably enforced. While it is generally recognized that most soluble foreign substances may be toxic to aquatic life in sufficiently high concentrations, but harmless when adequately diluted, these regulations usually do not indicate just what concentration, if any, of such waste substances, short of those known to have caused heavy fish mortality in a stream, may be deemed entirely safe or permissible or what reasonable criterion may be applied in arriving at such a decision.
2. Industrial concerns are entitled to advance information concerning the degree of improvement of their harmful wastes that would be judged entirely satisfactory, before the installation of costly waste-treatment facilities and other improvements are undertaken.
3. The increased attention to biological aspects of pollution problems and the interest in toxicity tests shown recently by many industrial concerns and organizations have been gratifying and should be promoted and encouraged.
4. The success of such regulations or standards experimentally adopted by the Louisiana Stream Control Commission for the control of the disposal of pulp and paper mill wastes into certain streams, in which fish mortality formerly had occurred repeatedly in spite of earlier attempts to regulate the waste discharges in other ways, can be cited as evidence of their practicability and desirability.

D. The following are some of the objections to the adoption of regulations or standards of the above-mentioned type which frequently have been raised:

1. Toxicity evaluation methods have not yet been, and for various reasons probably cannot be strictly standardized like chemical and bacteriological tests now in common use.
2. Available technical personnel in industrial laboratories, being untrained for such work, would find the toxicity tests very difficult to undertake, and the results often would not be entirely reliable and reproducible.
3. Bioassays are useful only for the measurement of the pollutional strength of an effluent before its discharge, and are not applicable to the measurement of the extent or persistence of moderate pollution in the receiving stream, and of the degree or rate of its natural purification.
4. Exact duplication in the laboratory of the actual, complex stream conditions which may influence the toxicity of wastes is not possible, so that the bioassay results may not always be strictly applicable under these conditions.

E. The above objections or arguments can, however, be answered as follows:

1. No other known way to define and control harmful pollution with toxic wastes is as sound and generally satisfactory as that which is advocated here, or offers more promising possibilities for further development, refinement and increased standardization.
2. The proposed bioassays are not actually much more difficult to perform than many routine chemical and bacteriological tests,

if adequate instructions are provided, and more trained biologists can and should be consulted and employed by industry in solving its waste disposal problems.

F. The application of comprehensive waste disposal standards to the control of pollution of streams receiving wastes from many different sources involves many complications and difficulties, but is not deemed impossible, especially if a new basic principle can be widely accepted or agreed upon:

1. The total volume or flow of a receiving stream could reasonably be apportioned among the various users, each of whom would be entitled to the use of only his fair share (determined without reference to the nature or quality of wastes discharged into the stream by the other users) for adequate or safe dilution of his wastes, in accordance with a set of comprehensive standards.

2. There is evident need for some reasonable compromise between the extreme views of those who believe that all public waters must be brought back to a state of pristine purity, and those who insist on maximal utilization of any stream's waste-disposal capacity for any given type of pollutant, without much regard to the complex and generally unfavorable interactions between the effects of the various types of pollution. The present proposal involves such a compromise.

G. Some standardization, or greater uniformity, of toxicity evaluation procedures would increase the usefulness of the bioassays greatly, and a start has been made toward this end.

1. Methods of determination of the toxicity of various chemicals and wastes, and of expression and reporting of the results,

which have been adopted in the past by different workers, have been too variable and often defective or not suited to practical application, so that data reported in the literature often are not at all comparable, or are even quite misleading.

2. Among recent attempts to promote uniformity of these procedures, as well as to instruct inexperienced workers in the performance of reliable tests and the interpretation of results, has been the publication (by the Atlantic Refining Co., Philadelphia, Pa.) of a limited, preliminary edition of a treatise or manual entitled "The Evaluation of the Toxicity of Industrial Wastes, Chemicals and Other Substances to Fresh-Water Fishes" by W. B. Hart, P. Doudoroff and J. Greenbank in 1945 (For reviews see Sewage Works Journal, March, 1946, Vol. XVIII, No. 2, pp. 274-275 and pp. 350-352). It is hoped that this will lead to the publication of a revised and more widely available and useful manual.

H. Various aquatic organisms can be useful as test material, or as "bioreagents", for toxicity bioassays, but some are outstandingly suitable for practical work.

1. Fresh-water fishes, particularly the more valuable native species, probably are the most satisfactory test material, by and large, for the following reasons:

   (a) Fish life is the most valuable biological resource directly affected by stream pollution with toxic wastes.

   (b) Test results obtained with fish as test animals are deemed most significant, relevant and acceptable by the layman.

   (c) Fish mortality is probably the most common cause for public complaints about pollution.

(d) Fish are quite sensitive to most toxic pollutants, and waters which are entirely favorable for the native fish life generally will support an adequate, diversified fauna and flora.

(e) While goldfish often can be very easily obtained, kept and used, and their use as standard test animals has been proposed by several workers, they are exceptionally hardy and otherwise atypical, so that they are probably less desirable for practical work than many native forms, such as sunfish, bass, trout and minnows.

2. The water fleas Daphnia also are useful test animals for the following reasons:

(a) They are extremely sensitive to many toxic substances.

(b) They can be easily bred in the laboratory, in order to provide a constant supply of material of known and uniform history, and because of their small size little space, water, etc., are required for their use.

(c) They represent an important group of fish-food organisms (Entomostraca), which should be protected, even though to most laymen they are unfamiliar and may be less acceptable as test animals.

I. Bioassay procedures and conditions must be chosen with due consideration for certain requirements of the test animals, the pertinent properties of substances tested and the intended use of the test results, and the experimental conditions must be carefully controlled. The following are some considerations of major import:

1. The water used in preparing test dilutions of a waste must be like that of the stream into which the waste is to be discharged.

2. The experimental temperature must be appropriate and fairly constant, because of its pronounced influence on metabolism, etc.
3. Adequate concentrations of dissolved oxygen in the test solutions must be maintained at all times.
4. Artificial aeration of test solutions (with compressed air, etc.) is not permissible, unless there is assurance that it will not influence their toxicity by driving off volatile substances, etc.
    (a) The influence of such aeration often is difficult to predict even when known chemicals are tested, because of complex chemical reactions that may occur in the test solutions.
    (b) Oxygen depletion resulting from the presence of oxidizable components of wastes tested, as well as the respiration of the test animals, tends to interfere with toxicity evaluations, but if the toxicity is fairly pronounced, this interference can be overcome by one of several appropriate means other than undesirable mechanical aeration.
5. Experimental animals should be thoroughly acclimatized to the environmental conditions (temperature, water quality, etc.) under which the tests are to be performed in the laboratory, before the tests are undertaken.
6. Small fish are generally preferable to large ones as test animals, because they are more readily handled, require less space and consume less oxygen; and young fish tend to be more sensitive to various harmful substances than adults.

J. The "median tolerance limit", defined as the concentration of a waste or other substance tested at which just 50 per cent of the test animals can survive for a given, specified period of time (e.g., 24, 48 or 96 hours), or indefinitely ("ultimate median tolerance limit"), is believed to be the most satisfactory measure or index of relative toxicity or tolerance, and can be readily estimated with reasonable accuracy:

1. The median tolerance limit for any given period of exposure can be estimated by interpolation, after determining the percentage of survival (among a group of usually 10 or more test animals) at each of a few test concentrations which are lethal to less than 50% and to more than 50% of the test animals in that period of time.
2. The ultimate median tolerance limit (i.e., the concentration at which the 50% survival persists with indefinitely prolonged exposure) usually can be determined or estimated by extrapolation, after obtaining median tolerance limit values for a series of time intervals (usually logarithmic) and plotting these against the exposure time.

K. Inasmuch as any determined median tolerance limit is only a convenient, instructive and most readily reproducible measure or index of toxicity, and may not be regarded as a permissible or safe concentration of the substance tested in a natural aquatic habitat, liberal safety factors should be applied when the test results are used in connection with practical waste-disposal problems. In other words, the disposal rate of a toxic waste into a stream should provide for dilution several times (e.g., 3 to 4 times) that which was experimentally found necessary to ensure 50% survival of test animals after reasonably prolonged or indefinite exposure. This is required not only because it is desirable to ensure 100% survival of the test animal species (which usually is observed under experimental conditions at concentrations only slightly lower than the median tolerance limit), but also for the following reasons:

1. Younger animals than those tested and the various other species of aquatic organisms which must be protected may be much more susceptible than the experimental animals.
2. Seemingly sub-lethal concentrations of a toxic substance may nevertheless be harmful to the test animal species, interfering with normal growth, reproduction or resistance to disease, or may even directly cause mortality after very prolonged exposure, because of unexpected, long-time or cumulative effects.
3. Variable conditions in the natural environment occasionally may be less favorable for resistance to the toxic agent than the experimental conditions chosen.

L. While field tests wherein fish held in live-cars or cages are exposed to diluted wastes in the receiving streams sometimes are very instructive, especially when they are performed in order to supplement or check laboratory test results, toxicity tests made under controlled conditions in the laboratory generally can yield more satisfactory and useful results for the following reasons:

1. Precise control of waste concentrations tested, as well as of other environmental conditions, usually cannot be achieved in the field tests, so that the reliability and the reproducibility of results of the latter tests are often uncertain.
2. Lethal and highest possible concentrations of a waste often cannot be tested in the receiving stream, while tests only of normal, sub-lethal concentrations are of limited value, as they do not indicate what margin of safety is being, or should be, provided for. In the laboratory, on the other hand, any desired concentration can be tested readily and without endangering the fish population of a stream.

Plankton as Related to Nuisance Conditions in Surface Water

(Biological Problems Pertaining to Water Supply and Sewage Treatment)

(Dr. James B. Lackey)

Man has never been able to do without water very long. Preferably clear, cool, virtually tasteless and odorless; not like the "arf-a-pint o'water ---- green" that crawled and stunk, which Gunga Din gave the British Tommie. But, like the Tommie, we are grateful for even that kind if no other is available. Gunga Din's water must have been taken from a pool in full bloom, well exemplifying those nuisances which blooms can cause. For they all too frequently turn surface waters into nauseous soups of some green, brown or red shades that truly stink. Hence the reason for an article on "Plankton as Related to Nuisance Conditions in Surface Water."

Plankton may be defined loosely as suspended aquatic small plants and animals. A bloom is an unusually large number of plankters, usually one or a few species, per unit of the first few centimeters of surface water. An arbitrary definition has set 500 individuals per ml. as constituting a bloom. Extremely small algae such as Chlorella or Gleocapsa are not crowded at such an aggregation but Pandorina would be. There are, of course, plankters of which a single individual exceeds 1 mm. in diameter, or 1 ml. in volume.

Nuisances due to peak occurrences of these organisms are many. A partial list includes:

1. Nauseous tastes in drinking water
2. Nauseous odors in drinking water
3. Lakeshore decay with odor and debris
4. Interference with bathing, boating and fishing
5. Killing of fish
6. Prevention of stock watering

7. Shortening of filter runs in water purification plants
8. Effects on industrial water use, such as growths in cooling systems
9. Poisoning of waterfowl, stock, and possibly man
10. Poisoning of mussels
11. Secondary fish depletion because copper sulfate has been used to control blooms
12. Secondary increases in mosquito nuisances by increasing larval food

This list could be extended by specific instances. For example, during the war, a sewage plant effluent was turned into an otherwise dry stream bed near a small Texas town. The effluent, highly purified, produced heavy growths of filamentous algae which, in turn, produced pooling. In the stagnant waters, mosquitoes - some of them anopheline - bred abundantly. This is a highly specialized case and it might be questioned, academically, that either surface waters or plankton were concerned. The complaints of fishermen concerning the vast mats of filamentous algae in some Wisconsin lakes are similar, but there is no doubt that these algae can be planktonic or that they interfere with both bathing and boating as effectively as a bloom of Anabaena or Microcystis.

Tastes and odors in drinking water are only too familiar to water plant operators, as are shortened filter runs. Almost any group of algae may cause them, but blue-greens, brown flagellates (such as Synura or Uroglenopsis) and diatoms are the chief offenders. Increased operating costs accrue in such cases, but equally bad is the loss of revenue when summer cottagers leave because an onshore wind piles their beach with a loathsome mass of decaying algae. And according to newspaper reports the recent red waters near the Florida shores were simply a hugh bloom of dinoflagellates which not only hurt commercial fishing, but also left fish carcasses along shore in such numbers as to cause vacationists to leave.

Table 1

The number composition and occurence of blooms during a two-year survey.

	Number Samples*	Total No. blooms	Blue-Green Algae	Diatoms	Dinoflagellates	Cryptophyceae	Chrysophyceae	Volvocales	Green Algae (Other)	Flagellata	Ciliata
Mendota	59	22	2	10		3			6	1	
Menona	64	86	10	28		15	1	13	19		
Waubesa	64	112	12	52	1	18		16	11	1	1
Kegonsa	63	80	7	29		20	1	18	3	2	
Wingra	52	91	15	34		6	12	2	19	3	
Como	10	7		4			3				
Delevan	13	5		2		2			1		
Geneva	11	13	3	7		1			2		
LaBelle	13										
Lauderdale	13	1					1				
Nagawicka	13	1		1							
Nemahbin	13	2		1		1					
Oconomowoc	14	4	4								
Okauchee	13	1					1				
Pewaukee	12	6	1	4			1				
Rock Lake	12	5		1		2	2				
Crawfish River	10	20	3	12			1	2	2		
Koshkenong "	13	33	9	11		5	2	2	4		
Rock River	14	20	2	9			1	1	7		
Totals	478	509	68	205	1	73	26	54	74	7	1

It has been observed frequently that stock will not drink while a heavy red bloom of Euglena sanguinea is present. Stock deaths have been attributed to poisonous blooms in the U. S., Africa and Australia; and in late 1946 waterfowl deaths in Utah were ascribed to huge numbers of dinoflagellates. Mussel poisoning along the Pacific Coast is familiar, and its appearance along the Atlantic Coast should be carefully watched for, as we increase the species of shellfish we eat. There are numerous instances of enteric diseases in man, apparently waterborne, and not satisfactorily accounted for, but which seem to travel downstream - in one instance, at least, paralleling the downstream travel of an algal bloom.

What are the nature, occurrence and causes of these blooms?

Blooms are typical of shallow lakes in the late summer but may occur in almost any body of water at any time of year. They are not generally noticed by laymen until surface waters are obviously discolored. Blue-green algae are perhaps the most common producers of blooms in stagnant water, but in streams a much wider variety of organisms is encountered in bloom densities. Colors range from a rather pale yellow-green or blue-green such as caused by Anabaena in Prospect Park lake in Brooklyn in 1924, to yellow (Gleotrichia, Mendota, 1942), brown (Peridinium, Chillicothe, Ohio, 1936), or red (Euglena sanguinea, North Alabama, 1935; Azolla, North Mississippi, 1940). Table 1 shows the organism groups which reached bloom proportions (i.e., 500 organisms per ml. of raw water) - a total of 509 times during a two years' survey of 16 southeastern Wisconsin lakes and three rivers in 1942-43. It should be clearly understood that not all this impressive list of 509 blooms were of the nuisance type.

There were actually more blooms - 509 - than the 478 samplings. Evidently high populations in Monona, Waubesa, Kegonsa, Wingra, Geneva, and the three rivers were mixed, of two or more species, and their normal biota rich and varied. Except Geneva, all had nuisance conditions. Blooms in Geneva were due largely to very small organisms; the layman would never have noticed them. Mendota had one extremely bad condition - a particularly nauseous population of Gleotrichia.

The table also indidates to what group these abundant organisms belonged. The only animal blooms were flagellates and ciliates although no account of copepod populations was taken. Animals were not a nuisance. There were only 68 high occurrences of blue-green algae, generally the most troublesome group, while diatoms, rarely obnoxious, were high 205 times. Obviously the table indicates that any of the groups of algae may pass bloom proportions if conditions are right. No chronology is shown here, but these peak populations occurred at any time; two very dense swarms of Volvocales (Chlamydomonas and Diplostauron) occurred under ice cover in Kegonsa. Diatom abundance was noted throughout the year and in these lakes was due most frequently to small naviculoid forms and to bandbox forms such as Cyclotella; small, and not productive of color in surface waters. In rivers forms like Synedra occur in huge swarms, and overnight may make their presence felt in the filter runs. In Lake Michigan Tabellaria and Asterionella have been conspicuous. Diatoms, however, are rarely troublesome except to water supplies.

Dinoflagellates, while frequent in these Wisconsin Lakes, were not a nuisance. In oceanic waters their blooming is well known, and their oily inclusions could be very troublesome in water supplies.

Cryptophyceae have not been reported as troublesome, but because they sometimes occur in huge numbers, may contribute to tastes and odors. Centrifuging a virtually pure population of Cryptomonas produced almost no whole cells; but the olive-green slime adherent to the centrifuge walls was highly offensive in odor, and these organisms readily disintegrate on sand filters. They tend to be especially abundant in rivers. Chrysophyceae seem to occur in winter and spring or late fall. In this survey they were not troublesome, but are notorious to water works men. They are especially characteristic of non-calcareous waters; many genera, abundant along the Atlantic seaboard or in Lake Michigan, are totally lacking in the Ohio Valley. Volvocales probably rank next to blue-green algae as obvious trouble makers, and together with other green flagellates, such as Euglena, are often conspicuous in waters whose organic content is above normal. But they do not occur in such numbers as to form windrows on the beaches.

Other bloom-forming organisms such as Azolla, Lemna or small arthropod water fleas are either rarely troublesome or do not belong to the plankton.

The causes of blooms have long been debated. Highly special or local causes may occur at times, but there seems little reasonable doubt at present that one or more optimal conditions - light, temperature, pH food - produce them, and perhaps the most compelling of these is the nutrient content of the environment. Many laboratory studies have indicated that nitrogen and phosphorus relationships are perhaps the most critical. Trace elements should be sufficiently abundant in natural waters from large drainage areas to supply the infinitesimal amounts presumably needed. But nitrogen and phosphorus demands are comparably large. Drainage areas may supply these demands from four sources: Original, virgin land; agricultural (fertilized) land; sewage, trade wastes.

Table 2 shows inorganic nitrogen relationships in two small Ohio creeks draining small areas, these determinations were made when light and temperature were optimal for many species, and stream flows average. Both creeks showed small amounts of nitrites and nitrates but admission of a sewage effluent below Station 2 in Lytle Creek changed that condition. Station 1 was above the town, some of whose street drainage was responsible for the increase at Station 2. Within about four miles (Station 5) nitrates had decreased to normal, nitrites had dropped nine-tenths, and organisms had multiplied twenty-five times; there was a dense bloom of Euglena, Chlamydomonas and Phacotus.

Table 3 shows that temperature may keep populations low even if sufficient nitrogen and phosphorus are present; but rising temperatures result in a sharp increase of organisms, until the available nutrients are lowered past a critical point, when organisms also decrease sharply. This table does not indicate that most of the organisms in Lake Delevan in January and February were quite small, whereas in April and May the organisms were much larger and different species.

Detailed studies of these Wisconsin Lakes gave the following average inorganic nitrogen and phosphorus values over a year's cycle.

Table 2

Inorganic Nitrogen - Organism relationships in two small Ohio creeks.

Station	Nitrates p.p.m.	Nitrites p. p. m.	Number of species	Number of Organisms
Lytle Creek				
1	0.1	0.01	40	826
2	.44	.02	32	1,629
3	.8	.08	31	954
4	.08	.01-	22	1,097
5	.08	.01-	39	25,189
Crown Creek	.04	.01-	40	313

Table 3

NITROGEN - PHOPHORUS ORGANISM RELATIONS
IN LAKE DELEVAN

Date	Inorganic nitrogen in p.p.m.	Inorganic phosphorus in p.p.m.	Number of species	Number of organisms
January 24	0.87	0.07	21	819
February 29	.59	.035	26	361
April 8	.36	.025	32	996
May 9	.32	.03	34	1,826
June 6	.22	.01	24	184
July 31	.09	.01	33	223

Table 4

Lake	Inorganic N,p.p.m.	Inorganic P,p.p.m.	No. Blooms
Mendota	.17	.018	22
Monona	.33	.041	86
Waubesa	.79	.38	112
Kegonsa	.35	.33	80
Wingra	.26	.012	91
Koshkenong	.39	.019	7
Delevan	.31	.023	5
Geneva	.10	.01-	13
Como	.14	.01-	0
Lauderdale	.13	.01-	1
Pewaukee	.15	.01-	1
Nagawicka	.37	.016	2
Upper Nehmabin	.24	.013	4
Okauchee	.13	.01-	1
Oconomowoc	.12	.01-	1
Lac La Belle	.19	.01-	6
Rock	.10	.01-	5

On the basis of this and other data Sawyer reasons: "In general, it may be concluded that lakes showing average annual concentrations of inorganic nitrogen and phosphorus in excess of 0.30 and .015 p.p.m. respectively, will produce frequent nuisance blooms." Table 4 generally bears this out, but of course there are exceptions. Mendota produced one nuisance bloom in the two years' study unless the masses of filamentous algae be so called, despite its low nitrogen average. Wingra and Geneva, despite low phosphorus averages, produced many blooms. But virtually all of these were very small organisms, and the term bloom applied only when there were more than 500 of some species per ml. of raw water. No discoloration or nusiance would have been evident.

Positive results are equally evident in Monona, Waubesa and Kegonsa. Here a large amount of contributed fertilizing minerals came from properly functioning sewage plant effluents, although agricultural drainage and industrial drainage were also contributors. Blooms in these lakes were so abundant and so frequent as to evoke legislative action on the part of the lake shore residents. Most of the nuisance conditions here were due to blue-green algae; Microcystis and Anabaena being the chief offenders.

But there is far too little evidence as to the causes of specific swarming growths of organism. In shallow, warm lakes, accumulating nitrates and phosphates seem to produce blue-green algae, but agricultural drainage into streams of moderate hardness seems to produce swarms of euglenoid flagellates, small non-motile green algae and green flagellates belonging to the Volvocales. Sulfuric acid, drainage from exposed coal seams produces, particularly, Euglena mutabilis. Distillery wastes have shown most abundantly, Volvocales: the first United States record of Chlorobrachis gracilla was a bloom of them in whisky distillery wastes at Lawrenceburg, Indiana. Cedar swamp acid waters along the Atlantic coast have produced blooms of the green flagellate Gonyostomum semen at Woods Hole, in New Jersey and near Savannah, often with an accompanying huge population of Euglena polymorpha. Reservoirs in areas underlain by granitic rocks may be troubled by yellow-brown flagellates such as Uroglena, Uroglenopsis, Synura, Chlorodesmus and similar forms. Flagellates of these types are common to waters whose pH tends toward acidity and are almost lacking in waters which are prevailingly hard. The pattern behind many blooms is reasonably clear, but specific causes are not and causes probably are due to combinations of conditions. An accumulation of nutrients is certainly necessary. Small streams draining sparsely inhabited areas along the Columbia River had as small plankton populations as have been found despite summer temperatures and low, clear water stages. Positive evidence is found in the increased plankton populations of fertilized fresh water fish ponds, as well as marine experiments at Milford, Connecticut; Woods Hole; and lochs in Scotland.

Control of nuisance conditions has followed three general lines. In the Wisconsin lakes a sewage treatment plant was put into operation and control of some industrial wastes was initiated. Further control by copper sulphate was used. An attempt was made to secure legislative action for diversion of sewage plant effluent. Storm sewer wastes and agricultural drainage were neglected. None of these schemes achieves more than partial success. Treatment of sewage reduces the B.O.D., and by maintaining oxygen and decreasing putrescible matter, changes the nature of blooms from saprophytic organisms (mainly bacteria) to presumable altogether holyphytic ones. This probably decreases the nuisance, but leaves an abundance of available N and P in food form. Copper sulfate treatment is temporarily successful, but since copper accumulates in the bottom mud where it apparently destroys clams, worms and insect larvae, its long continued used is questionable. The same question should be raised for recently devoloped algacides of other natures which upset biologic balance. Diversion of sewage or sewage plant effluents from a lake to a stream merely begs the question. The stream near Madison are already extremely rich in plankton, and additional food might cause nuisances therein.

Control or elimination of bloom nuisances thus far is purely temporary. The most promising investigations would seem to aim at the removal of phosphorus. Mitrogen might at times be available by fixation from the atmosphere. But before phosphorus removal is attempted, further studies of the amounts and mechanism of contribution from agricultural drainage should be made. In some instances, as when fertilizer is spread on iced over fields, it appears the farmer is paying for fertilizer which, on the first thaw or rain, at once washes into streams or lakes. The biology of industrial wastes needs much more study. The wastes of tanneries have a B. O. D. averaging 1200 p.p.m.; those of milk anddistillery wastes are far greater. Many wastes such as those of paper and chromium plating industries are indirectly or directly toxic, even though they leave the oxygen content of water largely unaffected. Such toxic effects may likewise be nuisances. Prevention of pollution or poisoning of shellfish areas, bathing beaches, lakes, and streams is better than cures. And if the biochemist or chemical engineer can achieve prevention by recovery of useful by-products as an economic measure, very little legislation will ever be necessary.

Conditions of Life in Natural Fresh Waters

(P. Doudoroff)

1. This lecture is a brief introduction to limnology (in the broad sense, including rheology), the branch of science dealing with inland waters, with attention to their physical, chemical and biological features and their interrelations. An understanding of the factors determining the biological productivity of inland waters is the principal, integrating objective of limnological investigation.

II. Physical conditions and their biological significance:

   A. Temperatures in fresh-water environments:

      1. Temperatures in U. S. (temperate zone) stream and lake waters usually vary between 32° and about 90°F. (Or within a narrower range), and almost never exceed 98°F., this limited temperature range being related to the high specific heat, freezing point, latent heat of fusion and heat of vaporization of water. Accordingly, most aquatic organisms are not adapted to withstand much more extreme temperatures.

      2. Within rather broad limits, the metabolic rate, activity and growth and development rates of aquatic animals increase markedly with rising temperatures (e.g., oxygen consumption usually increases by 2 or 3 times with a 10°C. temperature rise.

   B. Density and viscosity of fresh water vary with temperature:

      1. Maximal density is at 4°C. (39,2°F.), so that deep waters tend to remain always at or near this temperature.

2. Viscosity varies inversely with temperature and influences the flotation of small organisms.

C. Thermal or density stratification of inland waters:

1. The yearly cycle in the deeper lakes of the temperate zone:

   a. The fall overturn or circulation period, with uniform temperature near 4°C. and no resistance to mixing by wind action.

   b. The winter stagnation period, with bottom temperature near 4°C., and colder, lighter water and ice cover at the surface.

   c. The spring overturn or circulation period, again with uniform temperature near 4°C.

   d. The summer stagnation period with pronounced thermal statification, as follows:

      (1) The warm, surface zone, or epilimnion, with uniform, high temperature and circulation due to wind action and mixing by convection.

      (2) The transition zone, or thermocline, with temperature falling rapidly with increasing depth.

      (3) The stagnant lower zone, or hypolimnion, with relatively uniform low temperatures.

2. Less pronounced vertical temperature gradients and incomplete or irregular stratification (temporary) often occur in shallow lakes and quiet pools of streams.

D. Movement of water in lakes and streams:

1. Movement due to wind action and convection currents are most

important in lakes:

a. Wind-driven and convection currents tend to mix surface and deeper waters. During the summer stagnation period they are largely (not entirely) confined to the epilimnion, there being surface and return currents (in opposite directions) above and below a "shearing plane," due to wind action.

b. Wave action has effects similar to those of turbulence due to current in streams (see below), especially in shallows along lake shores.

2. Currents and associated turbulence in streams tend to keep silt and other material in suspension, dislodge, destroy or fragment organisms not especially adapted for life in fast water, and bring food to sedentary organisms. Variations of current velocity markedly influence the nature of the bottom and of aquatic life present. . . .

E. Freezing of water and the action of ice:

1. The grinding action of ice driven by waves and currents, scouring action of ice rubble and lifting action of anchor ice formed about sheltering stones on a stream bottom are extremely destructive of aquatic life in some habitats during the winter.

2. Ice with snow cover greatly reduces penetration of light (required for photosynthesis) into water below.

F. Transparency, turbidity and color of water, in relation to light:

1. In pure water more than half of all solar energy is absorbed in the first meter of depth, the absorption of light rays being selective, with those at the red end of the spectrum absorbed more rapidly than those at the blue end. Surface reflection further reduces the amount of light under water.

2. The turbidity of natural waters, due to suspended clay, silt, microorganisms and other organic matter, and the color of such waters, due to dissolved or colloidal organic matter of plant origin, vary greatly and have an important influence on aquatic plant life and productivity by reducing the penetration of light and limiting the depth at which photosynthesis can proceed.

III. Chemical conditions and their biological significance:

A. Dissolved oxygen content of lake and stream waters:

1. The low solubility of oxygen, i.e., the small amount present in solution in water at the saturation point, as compared with the amount in the atmosphere, is noteworthy.

   a. The solubility at atmospheric pressure decreases with increasing temperature from 14.6 p.p.m. at 0°C. to 7.6 p.p.m. at 30°C., while the oxygen requirements of aquatic organisms for respiration greatly increase with the temperature rise.

   b. The high tension, or partial pressure of oxygen at these low concentrations explains its availability to organisms for respiration.

2. The sources of dissolved oxygen are the atmosphere (solution of atmospheric oxygen at the surface in unsaturated water) and aquatic plant life during periods of photosynthetic activity (liberation of oxygen in the presence of light, while CO_2 is consumed), which may cause supersaturation of the water.
3. Depletion of dissolved oxygen is due chiefly to oxidation of organic matter by bacterial action and the respiration of aquatic animals and plants. The plants respire at all times, and so cause depletion of oxygen during periods of darkness.
4. The distribution and large natural variations of dissolved oxygen are related to variations of temperature, light intensity, abundance of plant and animal life (microscopic and larger forms), natural contamination with organic matter, and to thermal stratification, circulation and turbulence of the water, etc.
    a. Because of very slow diffusion of oxygen, water movement is essential for its downward transfer from the surface. Therefore, during periods of stagnation oxygen often is nearly or entirely depleted in deep water (e.g., the hypolimnion), where little or no light penetrates, even when there is ample oxygen near the surface. Such "chemical stratification" tends to correspond more or less with thermal stratification.
    b. Oxygen may also be nearly or entirely depleted at all levels in some warm waters rich in plankton and other organic matter, especially during the night, and also

in cold water under a persistent cover of ice and heavy snow.

5. Dissolved oxygen requirements of aquatic organisms vary greatly, and increase with rising temperature.

   a. Many plankton and mud-dwelling bottom organisms have low minimum oxygen requirements, and some (protozoa, worms, etc.) can carry on an anaerobic existence, in the absence of oxygen, for long periods. Some animals (pulmonate snails, some insects) normally come to the surface to breathe air, and so require no dissolved oxygen.

   b. Some fish require less than 3 p.p.m. D.O., and many can survive for limited periods at much lower concentrations, especially at low temperatures, but it appears that a minimum of about 4 p.p.m. D.O. may be required for prolonged survival of some warm-water fish at summer temperatures (probably 3 p.p.m. or less at winter temperatures), and a minimum of 5 p.p.m. may be required for the maintenance of an abundant, varied and flourishing fauna of such fishes in healthy condition at summer temperatures. Trout and other "cold-water" fishes are adapted for active life at low temperatures, and therefore apparently require somewhat higher oxygen concentration than the "warm-water" fishes (at least 1 or 2 p.p.m. higher than the above values) at corresponding temperatures.

c. Limiting D. O. concentrations (e.g., as p.p.m.) for aquatic life are more nearly constant at varying temperatures than corresponding values expressed as percentages of saturation.

B. Free carbon dioxide content of surface waters:

1. Although its solubility in water is high, little free CO_2 is in solution at equilibrium with air, because of the very low partial pressure of CO_2 in the atmosphere. In flowing streams the concentration usually is well under 5 p.p.m. and rarely exceeds 10 p.p.m.

2. Some atmospheric CO_2 is dissolved by water deficient therein (with a "negative" CO_2 tension). Otherwise, the major factors tending to increase the free CO_2 concentration are those which cause oxygen depletion, and vice versa. Therefore, free CO_2 concentrations (or CO_2 tensions) in natural waters tend to vary inversely with oxygen concentrations, and may increase to as much as 50 or 100 p.p.m. in the stagnant zone (hypolimnion) near the bottom of statified lakes.

3. Free CO_2 is utilized by green plants in the process of photosynthesis, but is not indispensable, as half-bound CO_2 can be utilized. Only unusually high concentrations of free CO_2 are demonstrably toxic to fish and other aquatic life, but lower concentrations may cause a significant increase of their minimum dissolved oxygen requirements.

C. Bicarbonates and carbonates of calcium and magnesium:

1. Concentrations of these salts, which account for most of the hardness and alkalinity of natural surface waters, vary

greatly (mostly between 30 and 150 p.p.m. as $CaCO_3$, but often being much lower or higher), depending upon geological and other factors.

2. The bicarbonates are the principal buffer substances in natural waters, and they serve as a source of carbon dioxide (half-bound CO_2) for plant photosynthesis, thus contributing to the productivity of hard (alkaline) waters. These waters thus usually contain a large store of available CO_2, which is lacking in soft waters, and when this is depleted by plants, the increasingly alkaline condition of the water which results tends to accelerate the absorption of atmospheric CO_2 at the surface.

3. Molluscs require carbonates in the water, and some other organisms thrive only in alkaline, or only in acid waters, but generally speaking, fish and other aquatic organisms can thrive in waters of very widely varying total alkalinity and hardness.

D. The pH value of fresh waters:

1. The pH value of natural waters varies chiefly with the carbonate, bicarbonate and free carbon dioxide content, which have been separately discussed.

2. The pH of unpolluted waters usually falls within the range of 6.5 to 8.5, or thereabout, but sometimes may be as low as 4.5 or less (bog waters containing organic acids), or above 9.5

3. Fishes and other aquatic organisms can withstand large variations of pH within the extreme range indicated above, some occurring naturally at both extremes, but, as already

noted, some organisms are more restricted in their natural distribution, with relation to pH.

E. Total dissolved solids content and the osmotic pressure of inland waters

1. The osmotic pressure of the body fluids of fish and other fresh-water organisms being above that of most inland waters, large natural variations of the concentration of physiologically relatively inactive salts (chlorides and sulphates of sodium, calcium, etc.) and the total dissolved solids content of these waters (mostly between 50 and 500 p.p.m.) are without any very pronounced effect on the aquatic life, but unusually high concentrations may have harmful effects through osmotic or specific toxic action of the salts.
2. Most fresh-water fishes can be expected to tolerate non-toxic salt concentrations osmotically equivalent to as much as 5000 to 10,000 p.p.m. of sodium chloride.

F. Nitrogen and phosphorus compounds in natural waters

1. Small and highly variable amounts of combined nitrogen (totalling a small fraction to a few p.p.m.), present in the form of inorganic compounds (nitrates, nitrites and ammonia) and in organic combination, and usually even smaller amounts of phosphorus, are of great importance to the nutrition of aquatic life.
2. Inorganic nitrogen, especially nitrate (although ammonia and nitrite also can be utilized directly or after conversion to nitrate by bacterial action), and phosphorus (phosphates) are essential nutrients for green plants, the growth of which often may be dependent on their availability.

3. Soluble organic nitrogen (found to exceed in amount the suspended organic or "plankton" nitrogen in some Wisconsin lakes studied) probably is utilized by both some plants and animals. Plankton nitrogen is consumed by animals.

4. Unusually high concentrations of ammonia (above 2 or 3 p.p.m.) may be harmful to aquatic life by toxic action.

IV. The large variety of inland aquatic environments, each of which has a population or association of plants and animals adapted thereto, but which differ in their productive capacity, is noteworthy.

A. The hydrographic instability of many stream environments and the destructive effects of floods, droughts, etc., on the stream biota adversely influence stream productivity.

B. Still waters (lakes, ponds, backwaters) tend to be more productive of plankton (i.e., small drifting organisms), and generally, than rapidly flowing waters.

C. However, moderately swift riffles with bottoms of coarse rubble, which provides good shelter, may be very productive of bottom-living organisms (various insects, etc.) which are especially adapted for life in such habitats.

D. Bottoms of silt, etc., in quiet water also may be very productive of bottom-living organisms (including many burrowing forms), which may serve as fish food. Weed-beds also provide favorable conditions for many organisms.

Conditions of Life in Polluted Waters

(P. Doudoroff)

A. Important changes of water quality which may result from various types of pollution and their biological effects:

1. Increased turbidity of the receiving water, resulting from the introduction of silt and various other suspended solids or the formation of precipitates through chemical reaction between naturally present and introduced dissolved substances, and also increased color due to colored wastes, reduce the penetration of light, and so adversely affect the photosynthesis and growth of green plants and the oxygen balance and productivity of the water. Healthy fish and other animals can withstand high concentrations of suspended solids, but extreme turbidities can be mechanically injurious to their gills, etc., especially in the presence of some mildly toxic dissolved substances.
2. Depletion of dissolved oxygen, due to bacterial decomposition of introduced organic matter or other oxidation reactions, may cause death of most aquatic organisms by asphyxiation, or, if less pronounced, may drive them away, decrease their resistance to disease and to various toxic substances, interfere with their normal development, etc. Oil films on water aggravate oxygen depletion by interfering with the solution of oxygen at the surface.
3. Increase of dissolved, free carbon dioxide, due to oxidative decomposition of organic matter and some inorganic chemical reactions, may stimulate the growth of aquatic plants under

otherwise favorable conditions, but may be injurious to animals when associated with pronounced oxygen depletion. Moderate free CO_2 concentrations such as are ordinarily encountered even in polluted flowing streams (under 15 p.p.m.) are not demonstrably harmful to most organisms under otherwise entirely favorable conditions (high D.O. values, etc.).

4. Changes of the pH value of water, due to the introduction of highly acid or alkaline wastes (also CO_2 production), may influence the solubility and toxicity of toxic pollutants (e.g., salts of heavy metals, etc.), and, if extreme, may be directly injurious to aquatic organisms. pH values below 4.0, or thereabout, encountered not too infrequently in streams heavily polluted with acid mine drainage, waste pickling liquors, etc., are generally lethal to fish and most other aquatic organisms, even in the absence of other harmful agents, causing injury to gills, coagulation of mucus and resulting respiratory failure, etc.

5. Increase of the total dissolved solids content, osmotic pressure and density of the water, due to pollution with various brines, etc., cannot often be deemed directly harmful to aquatic organisms, in view of their wide tolerance to natural variations of the salt content of inland waters and to high concentrations of properly balanced salts under experimental conditions and in some streams and lakes polluted with oil-field brines. However, excessive concentrations of salts can kill fish and other organisms by osmotic dehydration of their tissues, and lower concentrations of various salts can be lethal because of their specific toxic action, which varies with the nature and the combination of salts present. Also,

in some sluggish streams and shallow lakes polluted with brines, stable density stratification may occur, because of the tendency of the heavy polluted water to sink to the bottom and to remain there without mixing with the lighter surface water. In the absence of opportunity for reaeration, dissolved oxygen may be entirely depleted in the dense water near the bottom, and the bottom fauna (i.e., bottom-living fishes, insects and other fish-food organisms, etc.) destroyed by the lack of oxygen, if not by the high salt concentration.

6. Increase of ammonia, hydrogen sulphide and other toxic products of decomposition of organic matter may be injurious to aquatic life if excessive, especially under otherwise unfavorable conditions resulting from organic pollution.
7. Toxicity of the water, due to any of a large variety of highly toxic organic and inorganic substances that may be introduced as components of various industrial effluents, may be rapidly lethal to some or nearly all aquatic organisms, or may only interfere with their normal, healthy growth and reproduction. The initial, direct injury to the organisms may be largely external or internal.
8. Increase of plant nutrients, namely inorganic nitrogen (especially nitrates) and phosphorus, resulting from the complete decomposition and mineralization of organic pollutants, etc., may greatly stimulate the growth of green plants, and so also favor the production of animal life.

9. Increase of dissolved and suspended organic matter favors the growth of saprophytic plants and animals (molds, protozoa, etc.) which can utilize it as food.

B. Changes of the nature of the stream or lake bottom, due to pollution, such as blanketing of the bottom with silt, carbon fines, various precipitates, organic sludges, oily deposits, pulps, sawdust and other solids, also may have important biological effects, making the bottom unsuitable for its normal fauna (e.g., various bottom-living fish-food organisms), for the successful spawning of some fish, etc.

C. Horizontal, vertical and seasonal variations of pollution effects are related to a variety of hydrographic factors, such as rate and volume of stream flow, natural turbidity, temperature, thermal or density stratification, etc., all of which must be considered and understood in making or interpreting field observations of polluted waters.

D. The natural self-purification of polluted streams is due to physical, chemical and biological factors or processes:

  1. Non-biological factors include surface aeration, sedimentation, chemical oxidation, reduction, coagulation, precipitation, etc.
  2. Biological factors include bacterial digestion of organic matter and its mineralization or stabilization by biological oxidation, reoxygenation of the water and the utilization of inorganic products of bacterial activity (CO_2, etc.) by algae, higher aquatic plants and chlorophyll-bearing protozoa, and the consumption of organic matter, bacteria and other small organisms by protozoa and higher animals, which in turn are fed upon by larger forms.

E. The zones of organic pollution and self-purification, which represent, or are characterized by, different degrees of pollution or successive stages of self-purification of water receiving organic wastes, and which appear in longitudinal series and are usually readily distinguishable in a flowing stream below a point of concentrated pollution, in spite of considerable intergradation, have been variously defined and designated by different authors.

1. The following table presents and facilitates comparison of some of the older and widely used systems of separation and nomenclature of these pollution zones:

Authors	Suggested zones (Self-purification progresses to the right)			
Whipple, Fair & Whipple 1927	Degradation	Active Decomposition	Recovery	Clean Water
Suter & Moore 1922	Recent Pollution	Septic	Recovery	Clean Water
Forbes & Richardson 1913	Septic	Polluted	Contaminated	Clean Water
Kolkwitz and Marsson 1911	Polysaprobic	Mesosaprobic		Oligosaprobic

2. In the Ohio River Pollution Control Report of the U. S. Public Health Service (1944) the following system of classification of waters, or designation of pollution zones, was suggested (by F. J. Brinley) as being most broadly applicable in the area under consideration:

Zone I: *Zone of heavy organic pollution*: Dissolved oxygen not above 3 p.p.m.; water tending to be acid; plankton volume variable, not great, consisting largely of pollution forms; fish mostly absent, but some rough fish and other tolerant forms may be present.

Zone II. *Zone of intermediate pollution*: D.O. between 3 and 5 p.p.m. in the daytime; pH slightly higher than in zone 1: plankton volume greater, pollutional forms still predominating, but more chlorophyll-bearing forms, including some clean-water and intermediate forms, being present; fish more abundant and variable, but often deformed or parasitized.

Zone III. Fertile zone: D.O. subject to large diurnal fluctuations, but generally not below 5 p.p.m., and often well above the saturation value; water tending to be somewhat alkaline; plankton volume great (over 1,000 and usually several thousand p.p.m.), green forms of the intermediate group predominating, though pollutional and clean water forms also may be numerous; fish variable, plentiful and healthy, including many market fish.

Zone IV. Game fish zone: D.O. above 5 p.p.m., and usually near saturation values, water neutral to alkaline; plankton usually 300 to 1,000 p.p.m., clean-water forms predominating, and pollutional forms being very scarce; fish fairly plentiful, game and forage fish predominating, and various market fish, but relatively few coarse fish, also being present.

Zone V. Poor fish zone: D.O. and pH as in zone IV, excepting some streams polluted with acid (acid mine waters, etc.), where the water may be quite acid; plankton usually less than 300 p.p.m., consisting almost entirely of clean-water forms; fish largely of the game fish type and not abundant, because of natural conditions (lack of fertility) or acid pollution, which may be so severe as to eliminate all fish life.

3. The various zones of pollution or stream self-purification, described by the different authors mentioned, may or may not all be present and clearly recognizable in a polluted stream at any given time, this depending upon the degree or intensity of initial pollution, various hydrographic conditions, the natural

fertility of the water, etc. Repeated or dispersed pollution with wastes from different sources scattered along a stream may interfere with the normal or typical succession of these zones, and pollution with toxic wastes may introduce additional complications.

F. Aquatic organisms are adapted in varying degrees and in various ways for life in polluted water, and therefore may sometimes serve as useful indicators of pollution or relative purity of waters in which they are found.

1. Organisms normally found in polluted waters demand, or thrive best in, a rich medium, are usually highly tolerant to various adverse conditions, such as dissolved oxygen deficiency and high concentrations of toxic products of organic decomposition, and often possess especially efficient respiratory mechanisms, or are able to carry on an active an aerobic existence for long periods of time.
2. Examples of organisms typically found in polluted waters are the water mold, Sphaerotilus natans, and the rat-tailed maggot, Eristalis tenax larva, both of which are found in the septic zone of badly polluted streams; sludge worms of the genus Tubifex (also Limnodrilus, etc.), found in badly to moderately polluted. water and, where they become very abundant, marking the approximate limit for most fish life; and blood worms, or red-colored larve of the midge genus Chironomus, also found mostly in moderately to badly polluted water, and often being very abundant in the upper zone of recovery.
3. Examples of organisms usually found in relatively clean waters are mayfly and stonefly nymphs, most fresh-water mussels and game fish, such as trout. The abundance of green plants also indicates

marked recovery from pollution, or fairly clean water.

4. Since many factors other than pollution can greatly influence the distribution and abundance of various aquatic organisms, and the typically pollutional forms developed and were present in suitable, natural aquatic environments before the advent of serious stream pollution, the usefulness of the so-called indicator organisms, or index species, is somewhat limited, and the evidence of pollution or water purity that their presence provides may not be deemed entirely reliable, unless it is supported by various other types of evidence. Only when they are abundant and decidedly dominant is the presence of pollutional forms truly significant in this connection.

Studies of the Higher Aquatic Organisms in Relation to Water Pollution

(Peter Doudoroff)

A. Major objectives of biological studies of the higher aquatic life of polluted waters, which should be made in connection with sanitary investigations of pollution:

1. To determine the extent and relative degree of pollution by means of biological indicators, including:
    (a) The higher animal plankton forms (e.g., crustacea), some of which are very sensitive to many contaminants in the water with which they drift.
    (b) Bottom-living animals, which tend to remain in one place, and the nature of which indicates the effects of variable or intermittent pollution over a considerable period of time.
    (c) Fish, which are generally abundant and varied only in waters of reasonably good over-all quality.
2. To detect and evaluate damage (or benefits) to commercial and sport fisheries, which often is the most economically important and striking result of pollution.
3. To determine the roles played by the higher organisms in the natural purification of streams, and thus to contribute to the understanding of this process. While the role of the larger animals (with the possible exception of sludge worms, which can be extremely aboundant, and which work over and ingest great

quantities of organic mud) presumably is minor, as compared with that of the smaller, lower organisms (the combined or total bulk and surface area of which are greater than those of the less numerous higher forms), it probably is not insignificant.

B. Methods used in the study of the higher aquatic life in relation to pollution:

1. Qualitative and quantitative sampling of populations in comparable aquatic environments affected by varying degrees of pollution and subsequent natural purification of the water, by means of limnological sampling devices such as:
    (a) The Ekman dredge for sampling silt or mud bottoms;
    (b) The Petersen grab or dredge for sampling harder bottoms of sand, small gravel, etc.
    (c) The square-foot stream bottom sampler for rocky bottoms of rubble, etc., in shallow riffles of fast streams.
    (d) The Wisconsin plankton net, and other types, for sampling the larger plankton organisms, including all the higher animal forms.
    (e) Seines and other types of nets (trap or fyke nets, gill nets, etc.) for qualitative or semi-quantitative sampling of fish populations.
2. Experimental studies of the activities of organisms and their ability to survive in the presence of various pollutants under controlled conditions in the laboratory, or in enclosures or cages installed in polluted streams. (The bioassay of toxic wastes and their value is the subject of the next lecture).

C. Some general considerations and principles which should be understood and borne in mind when interpreting field observations and data:

1. The entire observed biological complex must be examined and considered as a whole, along with chemical and physical data, in attempting to arrive at conclusions relative to the pollutional status or classification of an aquatic environment.
2. Inasmuch as factors other than pollution may cause large differences of the nature and abundance of organisms present in different, superficially similar environments, or at the same location at different times, great caution must be exercised in attempting to relate such observed variations of the biota to the effects of pollution under investigation. Comparison of data that are not truly comparable may be highly misleading.
3. A truly adequate quantitative sample of an aquatic population, such as of bottom-living fish-food organisms, even in a short section of a stream, often is very difficult to obtain, because of usually large variability of individual samples obtained at different sampling points. Therefore, a large number of samples should be taken at each "station" or location sampled, and no importance should be attached to observed quantitative differences between the over-all samples from two or more stations under comparison (and presumed to be comparable) unless these differences are very striking and consistently shown by the individual samples, or their significance has been demonstrated through statistical analysis of the data.

4. An abundance of aquatic organisms which may be deemed potential fish food is not always indicative of favorable conditions for fish life, as they may not be available to, and utilized by, desirable fish species. However, the presence of a normal, varied bottom fauna and higher animal plankton, similar to those of nearby unpolluted waters, indicates that any existing pollution probably is not harmful to fish life also.

5. The best index of good over-all water quality conditions, from the biological viewpoint, is the continued presence of a normal or abundant and varied fish fauna, including many young, as well as adult fish, and with practically all specimens in healthy, well-fed condition. The growth rate of fish, indicated by their size and age, which can be determined by experienced workers through examination of the scales, also can be instructive. It should be borne in mind, however, that some hardy species of fish may thrive, and various other types (particularly migratory species) may occur temporarily and may not be noticeably affected during the more favorable periods, in aquatic environments which, because of pollution, cannot permanently support a normal, varied fish population.

6. Serious damage to a fish population and interference with the natural purification process in a polluted stream can result from the disruption (e.g., by some specific toxic agent, etc.) of the so-called "aquatic food chain," which begins with non-living matter required for the nutrition of bacteria, green plants, etc.,

and which culminates in the large predaceous animals (such as game fish), which feed on smaller carnivorous or herbivorous animals. The destruction of most of the algae, or the planktonic crustacea (entomostraca), or the bottom-living insect larvae, etc., would involve such serious disruption. On the other hand, it should be noted that the "food chain" as a whole is more adaptable to changed conditions than many of the individual species which may be regarded as "links" in this intricate chain, but which, if destroyed, often can be "bypassed," or replaced by other similar forms with similar food habits, but better adapted to the changed environment, so that the "food-chain" remains essentially intact.

D. Common causes of heavy mortality and depletion of fishes in polluted waters (reviewed and amplified):

1. Asphyxiation due to oxygen depletion appears to be the most common cause of mass mortality of fish, and less pronounced but chronic oxygen deficiency evidently is also a major factor limiting fish populations in polluted waters. The sudden, mass mortality often observed may result from:

   (a) The sudden discharge of unusual and excessive amounts of oxygen depleting organic wastes into a stream, or the flushing of disposal canals, swampy areas, etc., in which such wastes accumulate, by run-off after a heavy rain, causing a mass or wave of oxygen deficient water to appear and move downstream, trapping fish in the less rapidly affected areas (inlets, etc.) along the banks.

   (b) The sudden rising or "upwelling" of stagnant, deep water, in which dissolved oxygen has been gradually depleted by pollution (or natural causes), and its mixing with (or disdisplacement of) better oxygenated surface water, at the onset of deep circulation in a lake or stream after a period of prolonged thermal stratification and stagnation. (As an example, the trapping and mass mortality of fish only in a shoal area along the windward shore of False River, an "oxbow" lake in Louisiana, into which moderate amounts of domestic sewage were being discharged nearby, is cited. In this case, sudden cooling of the atmosphere and of surface water in the fall, together with a steady wind, evidently resulted in an off-shore surface current and a shoreward and upward return current, whereby formerly stagnant and oxygen-poor bottom water was brought to the surface first at the edge of the shoal, trapping the fish in the shoal area between this edge and the bank, and then killing them as the fresher water there was gradually replaced with the stale, deep water).

(c) Extreme diurnal or other fluctuations of dissolved oxygen, due to excessive fertilization of the water and consequent overabundance of both living and dead plankton and vegetation whereby oxygen produced during periods of bright daylight may be entirely depleted during periods of darkness or of inadequate illumination. (The case of a small, deep pond in Louisiana, in which the water had been excessively fertilized with commercial fertilizer, but was not otherwise contaminated, is cited as an especially pertinent example. Great fluctuations of dissolved oxygen at the surface and recurrent fish mortality here evidently were related to an excessive growth of plankton, as well as to variations of illumination, weather conditions and intermittent circulation of the water. Heavy mortality of fish during the night or early morning in very fertile lakes and ponds is not an uncommon phenomenon).

2. The toxic action of various industrial and mining wastes and the osmotic action of concentrated brines (e.g., oil-field brines) discharged into streams and lakes also may cause extensive fish mortality and depletion. A few examples of toxic wastes are electroplating and other wastes containing cyanides; wood-product wastes such as sulphate pulp mill wash-waters and pyroligneous acid produced by destructive distillation of wood (both of which are highly oxygen-depleting, as well as toxic); waste creosote and crude oil, which have highly toxic water-soluble fractions; acid mine waters, etc., etc. Toxic substances may increase the susceptibility of fish to oxygen deficiency, and vice versa.
3. The destruction of spawning areas by silting, sludge formation, etc., may reduce some fish populations by interfering with reproduction.
4. Interference with the growth of plankton and bottom foods by excessive turbidity, some toxic agents, blanketing of rich bottom areas with mining silt, organic or oily sludges and heavy brines, etc., may seriously affect the fish population of polluted waters indirectly, by reducing the food supply, without causing any demonstrable direct harm to the fishes present.

Analysis of Industrial Wastes

(M. B. Ettinger)

I. No comprehensive or universal routine is satisfactory for industrial wastes which may be encountered.

II. Mineral wastes in general are analyzed by inorganic analysis for particular constituents of the waste.

Examples

- (a) Chlorides in brine wastes
- (b) Iron, aluminum, manganese, acidity and sulfate determinations on acid mine wastes

III. Biological wastes in general may be satisfactorily evaluated using criteria usually available for sewage

1. Unmodified sewage procedures usually work well on food processing wastes. Deviations from sewage techniques may be caused by high or low pH's.
2. Wastes from processing biological materials may require considerable modification of technique. Modifications may include for example:
    - (a) Neutralizing and seeding before B.O.D.'s are determined
    - (b) Use of electrometric measurements for pH or end point determination

IV. With the other three types of organic wastes, the use of particular chemical criteria may be advisable.

1. No fixed routine may be set.
2. Examples of particular criteria.
    - (a) Phenol
    - (b) Oil

3. Certain criteria find general usage even with this category of wastes.

   (a) Solids, pH, alkalinity or acidity

   (b) Oxygen consumed

   (c) B.O.D. when skillfully executed and interpreted cautiously

   (d) Odor and taste potential, particularly after chlorination

V. Types of Industrial Wastes

(See attached table)

Types of Industrial Wastes

A. Wastes Principally Mineral in Nature

1. Brine wastes
2. Mine drainage
3. Waste slurries
4. Mineral washing slurries and suspensions
5. Pickle liquor wastes
6. Plating wastes
7. Miscellaneous wastes from inorganic chemical manufacture

B. Wastes that Contain Principally Organic Materials

I. Hydrocarbon Wastes

1. Oil wells
2. Petroleum refineries
3. Styrene manufacturing plants
4. Copolymer rubber plants
5. Butadiene manufacturing plants
6. Natural rubber processing or reclaiming
7. Gasoline filling stations, bulk stations, garages, etc.

II. Miscellaneous Organic Chemical Wastes

8. Munitions plants such as T.N.T., tetryl and ammonium picrate manufacture
9. Pharmaceuticals (Synthetic)
10. Synthetic fiber plants such as viscose or nylon
11. Organic chemical manufacture

III. Phenolic Wastes

12. Gas plants
13. By-product coke plants
14. Chemical plants
15. Synthetic resin plants (phenol resins)
16. Tar, road oil and creosoting plants
17. Wood distillation plants
18. Dye manufacturing plants

IV. Biological Wastes

a. Wastes from processing biological materials and/or from biological processes.

19. Tanneries
20. Pharmaceuticals (Antibiotics, biologicals)
21. Alcohol industries (Breweries and distilleries)
22. Miscellaneous fermentation industries
23. Glue and gelatin plants
24. Wool scouring
25. Textile manufacture
26. Pulp and paper manufacture
27. Laundry wastes

b. Food processing wastes

28. Canneries
29. Meat packing
30. Milk and dairy products plants
31. Corn products plants
32. Beet sugar plants
33. Cane sugar plants
34. Fish processing plants
35. Other food processing and dehydration plants

Table I

Breakpoint Chlorination

(Wm. A. Moore)

In 1928 Howard at Toronto showed that the chlorine dosage of water should be increased with increased NH_3 content. Laux of Anderson, Indiana Water Treatment Plant, rediscovered this phenomena in 1939 and used the O.T. flash test as a means of determining the presence of free chlorine.

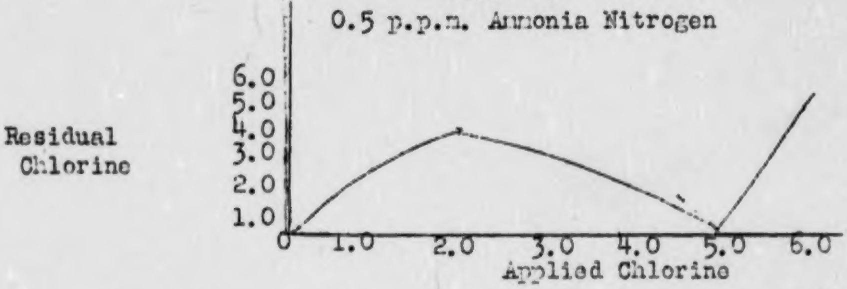
I. Laux found that the breakpoint was not constant but varied with variance in water conditions.

   a. Previously, workers did not know what chemical entities were present and water sterilization was based on presence of chloramines.

   b. Chloramines stable and residual chlorine as determined by O.T. could be obtained in water delivered to consumer.

II. Typical breakpoint curve between NH_3 and Cl_2 in a previously treated water to remove chlorine demand.

Slide I.



   A. Hump of curve comes at a chlorine-ammonia nitrogen ratio of 5:1.

      1. Corresponds to following reaction:

$$NH_3 + Cl_2 \longrightarrow NH_2Cl + HCl$$

14   71

$$71/14 = 5.07:1$$

         a. Other investigators have found this same ratio at hump of breakpoint curve.

   B. Chemistry of chlorination from hump to curve to breakpoint not as yet worked out.

      1. Havrill and others have postulated that N_2 is formed. Others have stated NCl_3, NO_2, etc, are among decomposition products of NH_2Cl. Based on formation of N_2 and Cl_2 : NH_3N ratio at breakpoint is 7.6:1.

2. Chapin working with high concentration of NH_3 (0.1 mole CH_3CONH_3 and 1000 - 2000 p.p.m. Cl_2) postulates at pH value above 8.5 only NH_2Cl is formed. Between values 8.5 and 4.4, a mixture of NH_2Cl and $NHCl_2$ is formed, the ratio of which depends upon the pH.

    a. Same reasoning does not apply necessarily to dilute solution of NH_3 and Cl_2.

III. Tests used in running breakpoint determination and chloramine-chlorine differentiation.

A. Standard O. T. test. Does not differentiate between chloramine and free chlorine. The standard ortho-tolidine solution uses the dihydrochloride as the compound can be obtained on a much purer state than ortho-tolidine.

B. Laux-Nickel O. T. flash test.

1. Uses less acidic O.T. solution.

    a. Water with low pH develops blue color.
    b. If pH is around 7.9 blue color changes to yellow.
    c. Higher pH values give yellow or orange color which does not change.

C. P-aminodimethylaniline: At pH 6.5, if free chlorine is present, red flash develops. If chloramine is present, then on acidification red flash develops.

Palin of England has placed this test on a quantitative basis.

D. Marks titrator. (Gold - silver electrode system). Free chlorine titrated with arsenite, than Kl added which reacts with chloramine to give Cl_2 which is titrated.

E. Oxidation - Reduction potential: Gold or Pt-Ir electrode is used against a standard calomel half cell. Electrode potential follows typical breakpoint curve.

F. Hallinan and Gilcreas O.T.A. test.

1. To cell A containing O.T. add sample. Mix and within 5 seconds add arsenite. Compare with standards. Result equals free chlorine.

    a. Within 5 seconds a part of the chloramine would have decomposed which would be counted as free chlorine.

2. To cell B. containing arsenite solution add sample, mix and add O.T. Read immediately (call reading B-1 and in 5 minutes call reading B-2). Value represents interfering substances.

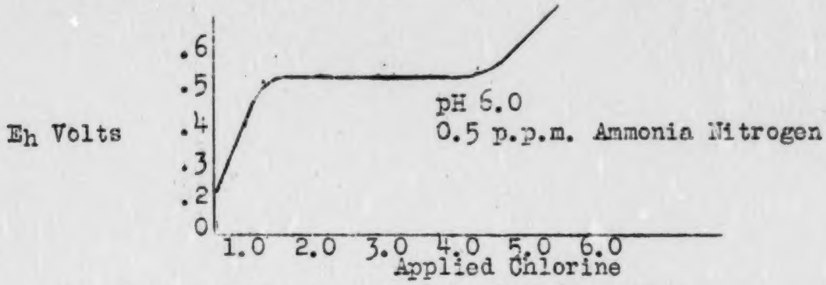
3. To cell O.T. containing O.T. reagent add sample, mix and compare in 5 minutes. Reading represents total residual chlorine.

O. T. - B.2 total residual Cl_2 (Call C)

A. - B.1 free available Cl_2 (Call D)

C. - D combined available Cl_2

IV. Effect of pH on breakpoint curve (H). At low pH small drop in potential obtained between hump and breakpoint.



1. Due possibly to reversal of the reaction

$$NH_3 + Cl_2 \rightleftarrows NH_2Cl_2HCl.$$

2. At all pH values sharp rise in potential curve occurs at breakpoint indicating presence of free chlorine.

   a. The flash test also confirms this. Flash test shows a + reaction at lower pH which also indicates a reversal of reaction between NH_3 and Cl_2.

V. Effect of time on NH_3 - Cl_2 reaction.

A. Regardless of contact time little change in NH_3 concentration until a Cl_2 - NH_3 - N ratio of 5:1 is reached at hump.

   1. NH_3 - N drops to 0 at breakpoint with no reoccurrence.

B. Cl_2 residual as determined by Marks titrator and O.T. give same type of curves.

   1. Little evidence of presence of Cl_2 and NH_2 Cl existing in same solution. As determined by quantitative p-aminodimethylaniline and Marks titrator methods.

C. Speed of reaction between NH_3 and Cl_2 shown in Table 2.

   1. Three minutes elapsed before flash test was made.

Table 2

Rate of reaction between chlorine and ammonia as indicated by results after 3 minute contact.

Cl-N Ratio	O-T p.p.m.	Flash pH 7.0	Acid
0.5:1	0.25	-	+
1.0:1	0.60	-	+
2.0:1	1.10	-	+
3.0:1	1.50	-	+
4.0:1	2.00	-	+
5.0:1	2.40	+	+

VI. $NH_3 : Cl_2$ ratio at breakpoint.

A. Table 1 shows maximum and minimum breakpoint ratios.

Table I

Minimum and maximum chlorine ammonia nitrogen ratios at breakpoint

	Minimum	Maximum
	8.4	9.2
	9.0	9.5
	8.0	9.0
	9.1	9.4
	8.6	9.4
	8.8	9.4
	8.8	9.2
	9.0	9.4
Avg.	8.7	9.3
Mean	9.0	

1. Minimum value is lowest point reached in E_h curve.
2. Maximum value is next value beyond breakpoint.

    a. Actual breakpoint occurs between these two points.

B. As pointed out before Cl_2 : NH - N ratio at hump is 5.07:1.

$$NH_3 + Cl_2 \rightleftarrows NH_2Cl + HCl.$$

14/71 + 5.07:1

C. Assume following reaction to take place from hump to breakpoint:

$$4\ NH_2Cl + 3\ Cl_2 + H_2O \longrightarrow N_2 + N_2O + 10\ HCl.$$

56 212.7

212.7 / 56 = 3.79

D. Then actual breakpoint should come at 5.07 +

3.79 = 8.86 : 1

1. Corresponds to mean breakpoint ratio found of 9.0 : 1.

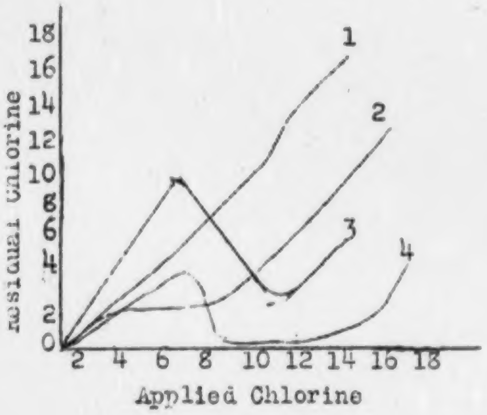
VII. Breakpoint curve actually encountered in practice do not necessarily follow typical NH_3 breakpoint.

1. Slide shows breakpoint of four different substances.
   - a. Egg albumin
   - b. Tryptose
   - c. NH_4Cl
   - d. Amino acetic acid

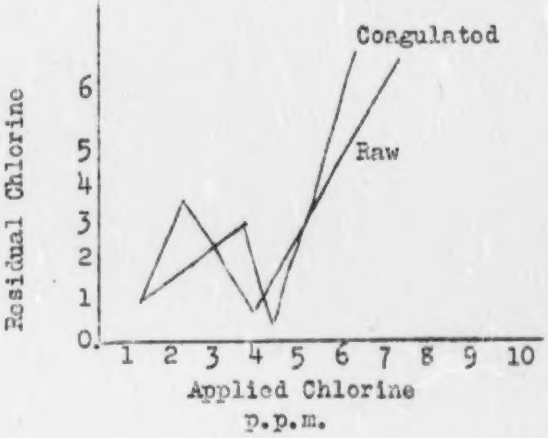
2. With egg albumin no typical brake is found. Tryptose shows flattening of curve at hump, then gradual rise. Amino acetic acid does show a hump and drop in residual Cl_2, followed by zero residual then gradual rise.

3. With ground waters Cl_2 : NH_3-N ratios as high as 25 : 1 may be encountered and degenerate breakpoint curve may result.

4. The removal of organic material by alum precipitation as shown in slide does produce a breakpoint curve similar to that of NH_3.



1 - Egg Albumin 2. p.p.m.
2 - Tryptose 2. p.p.m.
3 - Ammonium Chloride 2. p.p.m.
4 - Amino Acetic Acid 1. p.p.m.



CHEMICAL DETERMINATIONS FOR SEWAGE

(Frank Middleton)

__TESTS__: The ninth edition of Standard Methods for the Examination of Water and Sewage lists the following tests for sewage and sewage effluents, industrial wastes and polluted waters: -

Physical Tests - Temperature, turbidity, color, odor

Chemical Tests - Total solids, suspended solids and settleable solids, volatile and fixed.

Dissolved Oxygen, B.O.D. and relative stability.

Total, organic, ammonia, nitrite and nitrate nitrogen

Oxygen Consumed

Grease

Acidity, alkalinity and pH

Chlorides and Sulphides

Residual chlorine, chlorine demand

__Use of Tests__: These tests have been devised and are used singly or in combination for the following purposes:

(1) To give information on the strength, character and condition of the sewage to be treated or disposed of, and to aid in the selection of treatment and disposal methods.

(2) To measure and explain the performance of sewage treatment works and

(3) To determine and interpret or predict the behavior of waters into which sewage is discharged.

Grouping of Tests: These tests may be grouped as follows:

(1) Measures of concentration and state of the solids contained in the sewage and the appearance of the sewage; total, suspended and settleable solids, color and turbidity.

(2) Measure of the concentration, state and condition of the organic matter in sewage: Volatile total, suspended and settleable solids, B.O.D. relative stability, organic nitrogen, odor and oxygen consumed.

(3) Measure of specific substances or types of substances in sewage: Nitrogen in various forms, D.O., grease, chlorides, sulfides, acidity and alkalinity.

(4) Measure of the progress of decomposition of sewage: B.O.D., D.O., Nitrogen Odor, Sulfides, Temperature, and pH.

Collection of Samples: Fixed procedure cannot be prescribed due to variety of conditions under which collection must be made. Some important things to remember are:

1. Only representative samples should be used.
2. On sewage effluents.
   a. Collection over 24 hour period
   b. Sampling may be divided to represent shift work or to cover complete cycle of operation including all special variable or periodic discharges including Saturday and Sunday and clean ups. Particularly true of industrial wastes.
3. Individual portions should be collected in a wide mouth bottle of at least 35 mm diameter at the mouth and a capacity of 120 ml. Sample may be collected each half hour or at intervals of 5 min. and mixed at end of sampling time or combined in a single bottle.
4. It is usually essential to combine individual samples in proportion to the flow.

5. If it is necessary to collect "catch" sample the data must be interpreted accordingly.

Preservation of samples:

1. Samples of sewage or sewage effluent should be analyzed as soon as possible after collection.

2. Preservative must be chosen with regard to determinations to be made.

   a. Formaldehyde not recommended

   b. For D.O. samples the use of .7 ml conc. sulfuric acid and 1 ml. of 2% sodium azide is added to a 300 ml bottle and stored at the temperature of collection or in a 20° incubator under water seal. Or the samples may be used thru the iodine stage when collected. BEWARE EXCESSIVE USE OF ACID AND AZIDE. FUMES OF HYDROZOIC ACID FROM THIS CAN BE DANGEROUS AND WILL CAUSE EXCESSIVE HEADACHES IN SMALL AMOUNTS.

   c. B.O.D. must be run without preservative.

   d. Std. Methods recommends 1500 p.p.m. acidity to preserve bal. but will lower suspended and settleable matter and O consumed value. However, if the acid and the ammonia and nitrite are long in combination ammonium nitrite will be formed and eventually become nitrogen and water. A preferable method of preserving samples for nitrites and ammonia is by the use of sodium hydroxide in the amount of 1 g. per liter.

Physical Tests:

These tests are relatively simple:

Color reveals whether the sewage is strong or weak and whether it is fresh or septic and whether colored industrial wastes are present.

Turbidity is a rough measure of the amount of suspended matter.

Odor indicates whether sewage is fresh or stale and may detect the presence of certain industrial trade wastes.

Temperature measurements are necessary to the proper interpretation of chemical and biological reactions. The concentration of dissolved gases is influenced by temperature as is the rate of settling and percolation. Tests for solid matter in sewage measure concentration and physical state of the principal constituents of the sewage. It is of importance to know how much solid matter is in suspension, how much settleable and how much in solution. We need to know something of the portions of organic matter in the different states. Since organic matter burns an approximation of the amount present is determined by ignition of the dried solids.

Plant Efficiency Measurements:

Results of plant operation are measured by purification efficiency tests. We may want to measure

1. The overall efficiency of the plant or
2. The efficiency of each unit. The overall plant efficiency is usually measured by

1. Reduction of B.O.D
2. Reduction of suspended solids
3. Reduction in bacteria

Other tests to show the state of the purification process include ammonia, nitrites, nitrates, D.O. and oxygen consumed.

To effectively measure individual unit performance various tests are needed. In the primary tank the purpose is to remove settleable solids. The index to efficiency here may be the Imhoff cone test for removal of settleable solids which include readily settleable and solids not readily settleable. An indication of the non-settleable solids give information as to the load expected on subsequent treatment processes.

To show the internal condition of the primary tank tests for B.O.D., pH, and hydrogen, sulfide may be helpful. These tests help to indicate whether the sewage is turning septic or not; if removal of settled sludge is inadequate and the possibility of dead areas in the sludge compartments. The B.O.D. on the effluent from the primary tank determines the demand for oxygen to be supplied by subsequent oxidation units.

The reduction of B.O.D., suspended and colloidal solids by biochemical flocculation, precipitation and straining in the case of filters is effected in the oxidation units and the tests for B.O.D., suspended solids, ammonia, nitrogenous compounds and D.O. offer indices of the degree of purification and stability of the effluent produced.

Sterilization efficiency is measured by the reduction in bacteria which may include total and coliform organisms. The test for residual chlorine is the usual guide in operating the sterilization unit.

Certain types of sewage treatment call for special modifications of some tests and additional tests. For example D.O. is an extremely important determination in activated sludge plant control. The rapid utilization of the oxygen by the sludge in the mixed liquor cause interference in the ordinary methods of determining D. O. It is necessary to introduce an inhibiting agent. Such an agent has been developed containing sulfamic acid, acetic acid and copper sulfate. After settling and siphoning off the sample the azide modification may be used to get the D.O.

Another test of importance in connection with activated sludge is the Sludge Index. This test indicates the physical properties of the sludge as to concentration or compacting. It may be defined as the volume in ml. occupied by 1 gram of sludge after settling 30 minutes.

A more detailed discussion of some of the tests follows:

Solids: Solids may be determined in the following forms:

a. Total solids on evaporation. A 100 ml. sample is placed in a weighed dish and evaporated. It is then dried to constant weight at 103°.

b. Total Volatile and Fixed Solids - Total solids are ignited at 600°C in a muffle furnace. Mg. lost is reported as volatile solids and mg. residue as fixed sol.

c. Total Suspended Solids - A 50 to 100 ml sample is filtered thru a Gooch crucible. This sample is dried and weighed. For more rapid determination of suspended solids, especially mixed liquors in activated sludge the aluminum dish method may be used. An aluminum dish with perforated botton is used. Filter paper is used and suction is applied. Results are calculated as in other suspended solids. A photoelectric method for total sus. sol. has been developed also.

d. Volatile and Fixed Suspended Solids - Sus. solids are ignited at 600° in a muffle furnace and the loss is noted as volatile matter. The residue is the fixed suspended solids.

e. Dissolved Solids - May be determined by difference between total and suspended solids.

f. Settleable Solids - (1) By volume. A liter sample is placed in an Imhoff cone and settled 3/4 of an hour. Gentle stirring plus another 1/4 hour completes the test. The detention time of the tank under consideration may be used.

2. By Wt. Susp. solids are determined as usual. Allow a well mixed sample to stand 1 hour or the detention time of the tank. Determine suspended solids on supernatant. This is the non-settling solids. p.p.m. by wt. of settleable sol. = p.p.m. sus. sol. - p.p.m. non-settling solids.

Nitrogen

Ammonia N - Organic nitrogen or ammonia nitrogen should be tested within 6 hours after collection of the sample due to progressive ammonification by saprophytic bacteria. Direct Nesslerization is preferable to distillation as it avoids hydrolysis of urea and other organic compounds. The sample may be clarified with zinc sulfate and sodium hydroxide. Add Rochelle Salt to prevent cloudiness and then nesslerize. Temporary or permanent standards may be prepared. Ammonia free water must be used in making dilutions. It may be prepared by redistillation with Bromine and standing overnight or by the addition of chlorine in the proportion of 10 p.p.m. to 1 p.p.m. ammonia and subsequent boiling until chlorine disappears.

Organic Nitrogen - By Kjeldahl Method.. This method converts all the Nitrogen except nitrite and nitrate to ammonia. The sample is digested with concentrated sulfuric acid. Selenium in the form of Hengar granules is used as a catalyst. The digested sample is cooled and diluted with ammonia free water. The sample is made alkaline with NaOH and distilled into weak sulfuric acid. The excess acid is titrated and the nitrogen value computed.

Nitrates

Two methods are given in Standard Methods. The reduction method calls for treating the sample with NaOH and boiling to remove ammonia. A strip of aluminum foil is then placed in the sample and reduction allowed to take place 6 hours or overnight. The sample is then treated as any ammonia sample.

In some industrial wastes when high nitrites are encountered they may be removed by boiling with ammonium chloride at a pH of 7.5 followed by treatment with sodium hydroxide and aluminum and examining for ammonia.

The phenol disulfonic acid method consists of evaporating the sample (1 to 25 ml) to dryness and adding 1 ml phenol disulfonic acid. The sample is diluted and made alkaline with KOH and compared with standards prepared from potassium nitrate. Highly colored sewages must be submitted to a clarification procedure. Activated carbon and aluminum hydroxide are used. Limitation: In presence of large amounts organic matter some charring may take place with a resultant off color in the samples.

Nitrites

Nitrites should be determined on fresh samples. The method is colorometric and reagents are sulfanilic acid, a naphthyl-amine-acetate. A sodium acetate buffer is beneficial. Standards are made from sodium nitrite.

Oxygen consumed. - This determination is a measure of the carbon oxidized in organic matter. It does not measure the nitrogen in organic matter. The test is valuable in estimating strengths of certain trade wastes and may be used in conjunction with the B.O.D. test.

Tests at this laboratory and others have indicated the present method (the permanganate) method given in Standard methods is not altogether reliable. A method using dichromate has been proposed.

Advantages offered by the dichromate method include (1) better oxidizing agent, (2) more stable reagent (3) less loss of volatile constituents by using reflux method (4) better endpoint.

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Physical and Chemical Examination of Water

(Wm. A. Moore)

The above subject matter is rather broad and includes a wide variety of chemical determinations such as: alkalinity or acidity, turbidity, color, chlorides, pH, ammonia (free), nitrites, nitrates, organic nitrogen, solids, phenolic compounds, B.O.D., oxygen consumed, dissolved oxygen, etc. Of all of these determinations perhaps the most important is the last named.

In the determination of dissolved oxygen there are three factors to be considered.

1. Securing the sample.
2. Preservation of the sample.
3. Actual determination of dissolved oxygen.

Dissolved Oxygen

I. Sampling

A. Stream samples

1. Should be taken with samples so arranged that the D. O. bottle is filled at least three times.

B. Sewage plant samples

1. Sewage plant samples taken in 1 liter bottle containing copper sulfate-sulfamic acid inhibitor. Use 10 ml per 1 liter.

2. Inhibitor solution

a. 32 gram NH_2SO_2OH in 475 ml distilled water.

b. 50 gram $CuSO_4.5H_2O$ in 500 ml water.
Mix and add 25 ml glacial acetic acid.

C. Treatment of samples

1. Should be iced if they are taken from heavily polluted stream.
2. May be treated with Winkler reagents in field but if pollution is heavy, there will be a loss of iodine.
3. D. O. samples may be preserved by addition of azide and H_2SO_4.

a. 0.7 ml concentrated H_2SO_4 and 1 ml of 2% NaN_3 per 300 ml D.O. bottle. (Inhibitor of copper sulfate-sulfamic acid is just as effective).

In 1888 Winkler developed his method for the determination of dissolved oxygen and subsequently many modifications have been proposed to correct for interfering substances.

II. Substances that interfere with dissolved oxygen determination and corrective measures to be taken.

A. Nitrites

1. Rideal-Stewart method formerly used (early 1900).

   a. Work at this station has shown that this method even though effective in destruction of nitrites is actually detrimental if organic matter is present.

2. Alsterberg azide method originated in Germany in 1925, but not introduced in this country until 1939.

   a. Original method used the azide in mixture with alkaline-iodide reagent which on acidification reacted with NO_2 to Form N_2.

   b. Method as originated in United States used the azide in acid solution before Winkler reagents were added.

3. This was to prevent the reaction of iodine with organic matter in the final acidification which would give low values for D.O.

   a. Barnett and Hurwitz returned to original Alsterberg method and dissolved the azide in the alkaline-iodine solution. This requires less manipulation.

B. Ferrous iron (one of most difficult to correct). One p.p.m. of Fe// will give an apparent loss of 0.14 p.p.m. of dissolved oxygen.

1. So far Rideal-Stewart method is best available for ferrous iron. It must be, however, coupled with other treatment as the Fe/// formed will react with Kl in final acidification to liberate I_2.

   a. Potassium fluoride (40% solution), used to tie up the Fe/// iron formed.

      1. Should be added at rate of 2 ml per 200 p.p.m. iron. (Note) Sometimes difficulty is encountered in the decolorization of $KMnO_4$ with oxalate. May have to stand 5-6 hours. Decolorization can be accelerated greatly by addition of a drop of $MnSO_4$. However, this causes results to be off in many cases.

      2. Final acidification should be with H_2SO_4 in preference to H_3PO_4.

C. Organic Matter - Rideal-Stewart method valueless in presence of organic matter, giving low results. The loss in D.O. appears to take place during the preliminary treatment.

  1. Best method available is the short Winkler method (Thierault).

    a. Sample shaken for 20 seconds in presence of twice usual quantity $MnOH_2$, acidified without allowing to settle and titrated immediately.

      (1) Inhibitor solution mentioned previously is helpful in preventing loss of D.O. in presence or organic matter.

      (2) Azide should also be used as nitrites may be present.

      (3) Pomeroy and Kirshman Method.

    b. Substitutes NaI for Kl, using 900 g NaI and 400 g NaOH per liter. This solution is saturated and some difficulty may be encountered in keeping all the NaI in solution. Merits of this procedure:

      (1) Decreased loss of I_2 vapor by the increased concentration of NaI.

      (2) Decreased reaction of I_2 with organic material by increasing concentration of NaI.

      (3) Appears to give slightly sharper endpoint than regular Winkler procedure.

D. Ferric Iron

  1. Potassium fluoride best reagent to prevent interference with ferric iron.

E. Dissolved oxygen in presence of large amount of suspended matter or colloidal matter.

  1. Useful in highly turbid streams.

    a. 10 ml. of 10% alum solution added per liter followed by 1.0 - 2.0 ml of ammonium hydroxide. Allow floc to settle and siphon off supernatant in 300 ml D.O. bottles and proceed with Winkler method.

F. Sulfites

  1. Alkaline - hypochlorite method only one available and its value is questioned greatly, due to complexity of manipulation.

G. Physical methods

  1. Vacuum extraction

a. Simple apparatus - removes about 90% of D.O. so correction factor must be used.

2. More complex apparatus designed at this Station, but its extraction efficiency not improved materially.

Discussion

A second test mentioned previously is the oxygen consumed determination. Potassium permangnate has and still is the standard oxidizing agent used in Standard Methods. There have been, however, three other oxidizing agents proposed, namely, ceric sulfate, iodic acid and potassium dichromate. Of these the latter has been most used and many modifications of the method have been proposed. At this Station, the Adeney Dichromate Method has been used until a reflux method was developed.

This method consists in refluxing an appropriate amount of sample with 25 ml of .2500 N $K_2Cr_2O_7$ and 75 ml of concentrated H_2SO_4. The total volume is kept at 150 ml. After 2 hours the mixture is cooled and the excess dichromate titrated with .2500 N ferrous ammonium slufate, using ortho-phenanthroline ferrous complex as an inside indicator.

The reflux method is effective in the oxidation of sugars, phenolic compounds, some amino acids, branched chain acids, and alcohols. It will not, however, oxidize straight chain aliphatic acids. Straight chain aliphate alcohols are oxidized to the corresponding acid.

Studies on Biochemical Oxygen Demand

(By C. C. Ruchhoft)

In a study (soon to be published) of the velocity constants of dilutions of more than 50 sewage samples, it was shown that a considerable variation in k values was encountered. The k values at 20°C. varied from 0.04 to 0.29 with a mean value of 0.15. This variation in k was unpredictable and was independent of the origin of the sewage (i.e. military, hospital or domestic). A statistical comparison of the k values found in a series of two dilutions showed that the higher concentration of sewage statistically gave the higher k value, and the coefficient of variation in k values was greater in the lowest concentration series.

Careful studies of a number of series of dilutions of the same sewage showed that the B.O.D. values could be divided into two groups, one of which was consistent and the other inconsistent. Examination showed that each inconsistent value was one in which the oxygen demand equivalent of the nitrite nitrogen exceeded the probable error in the B.O.D. data. In other words, variations in k were observed to result from the unpredictable formation of small amounts of nitrite after incubation periods of three days or more in some series. The oxygen demand equivalent of the nitrite formed was so small that it could not be recognized by plotting the B.O.D. results.

To eliminate the nitrification variable, series of B.O.D. observations following incubation periods from 1 to 10 days were made on 45 sewage samples by a dilution technique which permitted determination and correction for nitrite formation on aliquots of portions used for oxygen utilization determinations. This technique permitted the derivation of oxidation velocity constants for strictly carbonaceous demands. The k values for the corrected carbonaceous B.O.D.'s. obtained on 45 samples ranged from 007 to .25 at 20°C. The frequency distribution for these k values showed a skew pattern with values between 0.16 and 0.20 occurring most frequently. It was found that with this technique of correction for nitrite formation similar k values for carbonaceous oxidation were obtained in Theriault Nichol's water, formula C water and bicarbonate water. Examination for the initiation of nitrite formation in the dilutions of these 45 samples showed that 4 were nitrifying by the fifth day and nineteen were nitrifying by the tenth day.

Data were obtained in this study which indicated that the value of k in a series of B.O.D. observations is dependent to a large extent upon the period of observation selected for study, and to a lesser extent upon the sewage concentration. The unimolecular reaction can be fitted quite satisfactorily to carbonaceous oxygen demands for any selected period of observation. Due to the flexibility of this mathematical treatment the k and L values obtained apply only to the specific time and conditions of observation. Consequently, k and L values obtained for a short period of observation have little extrapolative value and cannot be used to estimate demands for extended periods.

There results contradict the conception that there is a uniform velocity constant for sewage dilutions even at a constant temperature. They also indicate that nitrogenous oxidation is commonly initiated over a very considerable time period during the incubation and the general theory of clear-cut carbonaceous and nitrogenous stages is untenable.

The factors, besides temperature and pH which affect the B.O.D. rate in sewage and industrial wastes, include the complexity and concentration of the microflora and fauna, the composition and concentration of organic matter in the substrate, the presence of necessary accessory nutrients, and of toxic ions or compounds, and the supply of oxygen. For this reason, the B.O.D. determination must be very carefully standardized when applied to sewage and industrial wastes. The manometric or oxygen utilometer method may be used on undiluted wastes, or the standard excess oxygen dilution method, for diluted wastes. The choice of method depends upon the purpose of the study. The results of the two methods are not necessarily comparable, and it is not possible to extrapolate results on observations for one day or less to longer periods of observation. The dilution method will be most useful for the majority of wastes, but the application of blank seed sample demands, as correction factors, is an erroneous procedure and should be avoided.

Efficiency of Sewage Treatment Processes Summary

(Conrad P. Straub)

Three processes are available for treating sewage, namely: primary treatment, secondary treatment, and disinfection. Consideration must be given in addition to the disposal of solid material which is removed in primary treatment.

Primary Treatment

The purpose of primary treatment is to remove, insofar as possible, the solid particles present in the sewage by physical and mechanical means. Such processes as screening, grit removal, grease removal, and sedimentation are included in this category. Important aids to primary treatment include chemical precipitation, flocculation, flotation, removal of finely divided solids by aeration, and removal of grease by aeration or aeration and chlorination combined. Slow sand filters may be considered a primary process where sewage is applied directly.

Screens - According to Keefer for rack openings 1/2" to 1 5/8" in size the difference in amount of screenings removed was not very great. An average of 2 cu. ft. of screenings per m.g. of sewage were removed with removals ranging from 0.44 to 5.7 cu. ft. per m.g. For racks with an opening of 2" to 3" removals in the neighborhood of 0.57 cu. ft. per m.g. were shown. Data for 13 plants using fine screens indicated removals ranging from 3.3 to 32.6 cu. ft. per m.g. or an average of 14 cu. ft. m.g. Other sources report that fine screens may be expected to give removals of S. S. in order of 5 to 20%, reductions in BOD varying from 5 to 10% and bacterial reductions of from 10 to 20%.

Grit Chambers - Data on 40 plants listed by Keefer show that quantities of grit removed ranged from 0.33 to 13.0 cu. ft. per m.g. with an average of about 3.5 cu. ft. per m.g.

Sedimentation - Efficiency of sedimentation is influenced by tank dimensions, characteristics of the liquid, and characteristics of the sewage solids. Normal means of expressing efficiencies of units is to plot per cent reduction in SS or BOD against detention time. Other means of expressing efficiency would be to plot actual pounds of SS or BOD removed per sq. ft. of tank area per day against (1) pounds of SS or BOD applied per sq. ft. of tank per day (2) overflow rates in gallons per sq. ft. of tank area per day, or (3) gallons per ft. or weir length per day. Expressing these data as indicated above will require information relative to the actual flow in m.g. per day, size of tanks (length, width and depth), weir length in feet, etc. Many of these items are not usually reported when operating data are published making it difficult to attempt to correlate results based on these parameters.

Average removals to be expected from primary sedimentation have been reported to range from 40 to 75% for SS, from 25 to 50% for BOD, and from 25 to 75% for bacteria. Keefer's data from 27 plants indicate SS removals in the order of 39 - 83% and BOD removals of 21 to 59% for primary mechanically cleaned tanks. Preflocculation of the sewage results in an increase in the removal of SS and BOD. Removals resulting from preflocculation in addition to settling were 63.2 and 22.1% for SS and BOD respectively, as compared with removals of 53.2 and 15.6% for sewage settled without preflocculation. Results shown for 24 Imhoff tanks indicated removals of 33.6 to 75.7% for the SS (average 59.3%) and 11.1 to 72.0% (average 35.1%)

for the BOD. Fifty-six plants studied showed removals of 39 to 75% for SS and 48 to 52.2% for BOD.

Mahlie reports data on a comparison of the performance of Imhoff tanks against primary settling tanks. The data indicate a removal of 61.8 and 51.7% for SS removals in the Imhoff and primary settling tank, respectively, and BOD removals of 34.9 and 30.0%, respectively. However, the actual load applied in each case was 2.96 and 4.44 pounds of SS per sq. ft. of tank area per day, respectively. This shows that the load imposed upon the primary tank was considerably greater than on the Imhoff tank. Studies by Hubbell & Giles have indicated that a flared inlet followed by a gooseneck weir appeared most satisfactory as an inlet device.

Chemical Precipitation - Hendon presents the case for chemical treatment under four headings: (1) the need for a process intermediate between plain sedimentation and biological treatment to meet certain dilution requirements economically, (2) the need for a process which can be adjusted economically to seasonal and other variations in either load or available dilution, (3) the need for an intermediate process for reducing the load on existing overloaded biological plants, and (4) the need for methods of pre-treatment of industrial wastes entering sewage-treatment plants.

Chemical precipitation plants are reported to remove 60 to 90% of the SS, 50 to 85% of the BOD, and 40 to 90% of the bacteria. Keefer presents data on 12 plants and shows SS removals varying from 51 to 95%, (average 79.8%) and BOD removals varying from 41.1 to 86.0% (average 61.5%). Donaldson reporting on practices at Coney Island gives the following data: with chemicals the average removal of SS was 61% and BOD 51%, without chemicals these removals were 44 and 31%, respectively. At Minneapolis-St. Paul the addition of 12 p.p.m. iron increased the SS removal to 83% as compared to 73% for plain settling and BOD removal from 35% for plain settling to 55%. Data from 9 additional plants indicate removals in the order of 77 to 90% for SS and 60 to 87% for BOD.

The following results are available relative to a tray-clarifier installation at Springfield, Missouri:

	Original Clarifier	Tray Clarifier
Effective area - sq. ft.	2500	7600
Flow - mgd	3.5	nearly 10 (about half was sludge from final clarifier).
Aerage reduction SS	41.2%	61.0%
Detention period	2 hours	33 minutes
Overflow rate	700 gals/sq. ft/day	725 gals/sq.ft/day

Secondary Treatment

Trickling Filters - Efficiencies of trickling filters are influenced to a greater or lesser degree by many factors such as sewage characteristics; method of application; depth of filter bed; temperature; size, shape and kind of filtering material; dosing and rest periods; and type of underdrainage system.

Low rate trickling filters will be considered first. Streeter reports that trickling filters will remove from 30 to 40% of the SS, 80 to 85% of the BOD, and over 90% of the bacteria. When preceded and followed by plain sedimentation these figures are reported as 70 to 92% for SS and 72 to 95% for BOD. Data from 21 plants listed by Keefer indicate average removals of 34.5% for SS and 64.5% for BOD. Eleven years data at Worcester, Mass., show a positive removal of SS during only one year. BOD removals ranged from 64.9 to 80.2%.

Data have been recomputed and plotted with pounds BOD load applied per cu. yd. of filtering material versus pounds of BOD removal per cu. yd.

of filtering material. In some instances an increase in removal with load was indicated for several plants studied. Loads applied were below 1 pound of BOD per cu. yd. of material.

Removals in final tanks are reported by Streeter to be about 50 to 70% for SS and bacterial numbers. SS removals in the final tanks varied from 7.8 to 33% at Worcester. Data for three additional final tanks following trickling filters indicate SS removals of 42 to 50% and BOD removals of 22.2 to 39.1%.

There is little extended data on high rate trickling filters. The manufacturers of bio-filters report that BOD reductions for the single stage process, in which one filter and one clarifier is used, will amount to 50 to 60%: for single stage completed treatment (one filter and 2 clarifiers) the reductions will be in the order of 75 to 80%, and for two stage treatment (2 filters and 2 clarifiers) reductions will be in the order of 90 to 95%.

Two studies have been completed by the Cincinnati staff on shallow recirculating filters at Elizabethtown, Ky. and Centralia, Missouri. Various conditions of operation were employed. In the first and second series at Elizabethtown the filters were operated in parallel with recirculation ratios of 0.69 and 0.90, respectively. In the third series, the filters were operated in series and the recirculation ratio was 0.63. For the primary tanks the removals for the 3 series were as follows: BOD 49.3, 49.5, and 57.8%; SS 53.2, 41.2 and 46.4%, respectively. Using the method suggested in "Sewage Treatment at Military Installations" (NRC Report) removal values based on the formula

$$\frac{\text{Total load to primary - load in primary effluent}}{\text{Raw Sewage Load}} \times 100 = \%\ \text{Removal}$$

were 60.4, 60.9 and 65.3% for BOD and 71.7, 58.5 and 63.3 for SS, respectively, for the 3 series.

In the secondary system BOD removals were 51.8, 63.5 and 71.8%, SS removals 25.9, 29.8 and 44.3 respectively. These low values result from the operation of the secondary clarifier. SS removals by the secondary clarifier alone were 0.8, 18.1, and 30.2%; BOD removals 9.1, 20.9 and 19.6% respectively.

Plotting the results of the operation of the filters to show the relation between BOD load and BOD removal in pounds per cu. yd. of material, the data could be represented by a straight line with no definite indication of reduction in removal in the loading range of 5 - 6 pounds of BOD per cu. yd. of filtering material.

At Centralia the study was divided into 4 periods. The first period was one of normal operation and involved no recirculation. Percentage removals by the primary tank were 46.3 and 74.2% for the BOD and SS, respectively. The filter showed a reduction of 45% of the applied BOD load and the final clarifier reductions of 45 and 43% for the BOD and SS. Overall plant removals were 84% for BOD and 86% for SS. During the second period the final effluent was recirculated. The results were as follows: primary, 24.4 and 59.6% for BOD and SS, respectively; filter 42.6 and 10.7%, respectively; and final clarifier 59.3 and 53.4%, respectively. Overall removals with recirculation of final effluent were 84.1 and 82.9% for BOD and SS, respectively. Filter effluent was recirculated in the third period. Removals were as follows: primary, 44.1 and 67.7% for BOD and SS, respectively; filter 54.4 and 22.0%, respectively; final clarifier, 62.2 and 65.6%; overall removals, 89.5 and 91.1%, respectively. Final tank underflow was recirculated during the fourth period of observation. Results were as follows: primary, 50.3 and 68.5% for BOD and SS, respectively; filters

51.2 and 31.5% respectively; final clarifier, 74.2 and 63.4% respectively; overall 93.3 and 91.8, respectively. The efficiencies reported vary quite markedly with the method of operation and are rather high, being due primarily to the fact that, for high rate filters, the BOD loads applied per cu. yd. of filtering material were low, ranging from 0.47 to 1.35 pounds.

Activated Sludge - The efficiencies to be expected from the activated sludge process vary. The usual values listed vary from 80 to 97% for SS removal, 85 to 97% for BOD reductions, and in excess of 90% for bacterial reductions. Twenty-two plants listed by Keefer show average removals of 84.4 for SS and 83.7% for BOD. Data collected on 31 activated sludge plants showed removals as follows through the various units: primary, SS 5.2 to 73.8% with an average value of 42.6%, BOD, 3.3 to 62.2% with an average value of 37.1%; aeration unit and final tank, SS 48.5 to 97.8% with an average value of 87.5%, BOD, 73.0 to 96.6% with an average value of 88.1%. Overall removals were SS 77.7 to 98.7% with an average of 93.6% and BOD, 86 to 97% with an average of 92.8%.

A 16 mgd diffused air plant, a 1 mgd Simplex plant, and a 0.6 mgd bioaeration plant were constructed at the Davyhulme Plant, Manchester, England. Results of operation based on BOD reductions were as follows: high supply diffused air - 92.5%, low supply diffused air - 89.2%, Simplex - 93.6%, and bioaeration - 84.9%.

Attempts were made to show relationships between efficiencies of operation of the secondary unit and the BOD load, the pounds of BOD in aeration tank influent per day per 1000 pounds of SS in aeration tank per hour of aeration; between the pounds of BOD applied and removed; and between the sludge index and the pounds of BOD per pound of SS, but without success.

Special Processes - Some data are available on results of the Guggenheim process. These show removals in the order of 91% for BOD and 93% for SS at New Britain, Conn. Mohlman and Gleason announced results of 91% for BOD and 97% for SS in experimental studies at Chicago. In an installation at Raleigh, North Carolina, the city received a guarantee that 80% of the SS and BOD would be removed.

Tests conducted at Goshen, Indiana, by Hurwitz and Miller showed 62% overall removal of SS and 47% removal of BOD by settling followed by coarse upflow filter. The magnetite filter removed 23% of the SS and BOD reaching it, thereby improving the overall treatment by 12% in SS and 14% in BOD as indicated by samples below and above the filter. Final effluents of 4.4 ppm SS and 8.7% of BOD are reported for magnetite filters used as a final polish on bio filter plant effluent at Liberty, N.Y. The BOD removal through the filter amounted to 49% and SS removal to 67%. At Denver, the overall removal obtained by settling and filtering through magnetite filters was 84% for the SS and 59% for the BOD. At Minneapolis - St. Paul, the magnetite filters were responsible for removing 28.9% of the SS and 13.9% of the BOD coming onto the filter. Overall removals for settling and filtering were 92.5% for the SS.

Disinfection

Chlorination is used most frequently for disinfection. Results obtained generally indicate that up to 99% of the bacteria present may be removed.

Epidemiology of Water-borne Diseases

(Dr. John J. Phair)

A. No set pattern for epidemiology of water-borne diseases

B. Enteric pathogens

  1. Lesions in intestinal tract

  2. Epidemic due to mass contamination

  3. In fecal matter

C. Control of enteric diseases not possible by same methods because of different host reactions

D. Comparison of diseases for study

  1. Cholera

    a. History - Was endemic in India in ancient times. Spread from Asia to Europe in epidemic proportions in 1820 - 1823. An epidemic followed in Europe from 1850 - 62. The last big pandemic was in 1902. During the course of the latter three epidemics, cholera was introduced in the United States but was never able to survive because of better sanitation in this country. The disease has never existed for a long period in the temperate zones and has required reintroduction when cases developed.

    b. The Disease - The cholera vibrio was isolated by Cook in 1884 but at that time was not linked with the spread of cholera. The vibrio is short-lived outside of its normal habitat in the intestinal tract. Cholera causes death by dehydration and exhaustion of the patient. The vibrio parasite is poorly adaptable to conditions outside of the intestines.

c. Transmission - Primarily as a water-borne disease. Secondary transmission is from case to case or by food, milk and raw vegetables.

d. Mortality rate is quite high, approximating 50% in some epidemics.

e. Requirements of conditions for disease to be endemic.

   1. High birth rate
   2. People have to live close together
   3. Poor sanitary facilities

2. Typhoid Fever

a. History - Was known in biblical times and frequently confused with typhus fever until the nineteenth century. The bacillus was described in 1880.

b. The bacillus penetrates the intestinal tract and causes lesions. Mortality rate is about 10%. About 5% of the people who have had typhoid fever will retain chronic infection and will remain infected. Parasite is an obligate parasite of man and very well adapted to man. Man may have the disease and not show symptoms.

c. Transmission primarily by water, food, milk and by carriers from area to area. Industrialization made possible the spread of the disease by the grouping of people in industrialized areas.

d. Factors necessary for the condition of disease:

   1. Large population, high birth-rate and/or immigration
   2. Poor sanitation facilities
   3. People living close together

e. Rural typhoid may eventually disappear because of the infrequencies of different people utilizing a single well water supply.

3. Bacillary Dysentery

   a. History - This disease was known in ancient times and it was not until 1875 that there was a differentiation between amoebic and bacillary dysentery. There are several strains of dysentery and no cross immunity is developed.

   b. The disease - The bacillus remains in the enteric tract and does not invade the blood stream. The very young and very old seem to be the most susceptible. The bacillus is very adaptable to conditions and is an obligate parasite of man. The disease is more common in the tropics than in temperate climates.

   c. Transmission - Carried from case to case, by water, food or milk. There are many sub-clinical cases in middle age groups.

   d. Control - Proper control necessary is good sanitation in the home. Water, food and milk sanitation will not handle the problem.

4. Salmonella Food Poisoning - Many organisms incriminated and not too much is known of them.

   a. Food poisoning - The disease is caused by toxins produced by organisms upon their introduction into the body. They are introduced into body by contaminated food supplies. The toxin grows only in food. The organism capable of producing toxin is not a parasite of man but of animals. There is no immunity and apparently all ages are effected.

b. Control - By proper food sanitation

5. Summary and Discussion - The spread of diseases or infections through bathing beaches
   - a. Dubious transmission
   - b. Enteric pathogens possible
   - c. Ear infections caused by not drying ears thoroughly
   - d. The virus of Polio and others may not be effected by chlorination

*NOTE: This outline is reconstructed from notes of Dr. Phair's lecture as taken by Mr. Donald J. Schliessmann.

Governmental Functions in the Control
# of the
Interstate Spread of Diseases

(Albert H. Stevenson)

LEGISLATIVE HIGHLIGHTS AND CHRONOLOGICAL EVENTS

Constitution

Preamble - "promote the general welfare"

Article I under General Powers of Congress

"provide for the command defense and general welfare of the United States."

1798 (Act July 16, 1798, Ch 77 1 Stat L 605) "An act for the relief of sick and disabled seamen."

Resulted in establishment of Marine- Hospital Service.

20¢/mo. deducted from seamen's pay to finance costs of medical care.

1878 (Act April 29, 1878, Ch 66, 20 Stat L 37) "An act to prevent the introduction of contagious or infectious diseases into the United States."

Foreign Quarantine

Resulted in establishment of quarantine stations at ports of entry. Quarantine regulations made by S.G. but could not conflict with state regulations. Consular officers at foreign ports report to S.G.

1890 (Act March 27, 1890, ch 51, 26 Stat L 31) "An act to prevent the introduction of contagious diseases from one state to another."

Domestic Quarantine

Limited to cholera, yellow fever, smallpox, and plague.

1893 (Act Feb. 15, 1893, ch 114, 27 Stat L 449) "An act granting additional quarantine powers and imposing additional duties upon the Marine-Hospital Service."

Strengthened the foreign and domestic quarantine regulations, more uniformity, superseded state laws if they were found to be inadequate.

1901 (Sundry civil approp. act March 3, 1901, ch 853, 31 Stat 1137) $35,000 made available to PHS to build a hygienic laboratory "for the investigation of infectious and contagious diseases and matters pertaining to public health."

1902 (Act July 1, 1902, ch 1370, 32 Stat L 712) - An act which changed the name of the U. S. Marine Hospital Service to the "Public Health and Marine Hospital Service" to the "Public Health and Marine Hospital Service of the United States."

This act also provided for a "Conference of State and Territorial Health Officers."

1912 (Act Aug. 14, 1912, ch 288, 37 Stat L 309) "An act to change the name of the PH and MHS of the US to the "Public Health Service."

Under this law the PHS "may study and investigate the diseases of man and conditions influencing the propagation and spread thereof, including sanitation and sewage and the pollution either directly or indirectly of the navigable streams and lakes of the United States." Resulted in Cincinnati Station - for Water and Sanitation Investigations.

1913 (Act June 23, 1913, Ch 3, 38 Stat L 25) An Appropriation was made for the prevention of the interstate spread of disease. Appropriations have been made annually for the same purpose since.

At this time the high incidence of typhoid on Great Lakes vessels resulted in the adoption of the first Drinking Water Standard. Bacteriological Std: Plate Count 100/cc permitted

B. Coli 1 in 5-10/cc tubes positive permitted

1919 Formation of Interstate Sanitary Districts

PHS engineers assigned to assist in formation of state sanitary engineering division and in certification of water supplies used by common carriers.

Drinking Water Standards revised.

States handled inspection and survey of water supplies and reported to Surgeon General.

1925 Drinking Water Standards revised - more severe - more water supplies meeting requirements.

1930 (Act April 9, 1940, 46 Stat 150) - An act to provide for the coordination of the public health activities of the government. (PARKER ACT)

Provided for commissioning sanitary engineers, dentists, and pharmacists in addition to medical officers.

1930 (Act May 26, 1930, 46 Stat 379) - An act which changed the Hygienic Laboratory to a "National Institute of Health."

Provided for a system of fellowships in the institute. Authorized acceptance of donations "for use in ascertaining the cause, prevention, and care of diseases affecting human beings."

1944 (Act July 1, 1944, ch 373, 78 Stat 410) "An act to consolidate and revise the laws relating to the PHS." (P.L. 410)

Title III - Powers and Duties of the P.H.S.

A. Research and Investigations - relative to causes, diagnosis, treatment, control, and prevention of physical and mental diseases and impairments of man, including water purification, sewage treatment, and pollution of lakes and streams.

B. Federal-State Cooperation - accept state assistance in enforcement of quarantine regulations, assist states in suppression of communicable diseases, prepare and distribute vital statistics, make grants to states.

C. Medical care

D. Lepers - care and treatment

E. Narcotics Addicts - care and treatment

F. Biological Products (regulation of preparation and distribution)

G. Quarantine and Inspection

Control of Communicable Diseases - Make and enforce regulations necessary "to prevent the introduction, transmission, or spread of communicable diseases from foreign countries into the states or possession, or from one state or possession into any other state or possession."

Title IV - National Cancer Institute set up as a division in the NIH

1946 (Act July 3, 1946 ch 538 Stat 487) - provide for National Institute of Mental Health (psychiatric disorders)

1946 (Act Aug. 13, 1946, ch 958 Stat 725)

"Hospital Survey and Construction Act"

Provides grants to states for survey and construction of hospitals and public health centers - Federal Hospital Council

CURRENT REGULATIONS & PHS ACTIVITIES TO AUGMENT THEM

1. Medical Examination of Aliens (1917) (1946 - Part 34)

   At foreign consulate or at port of entry.

2. Foreign Quarantine Regulations (1946 - Part 11)

   Quarantinable diseases

   cholera - 5 days

   plague - 6 days

   smallpox - 14 days

   louse-borne typhus - 12 days

   yellow fever - 6 days

   Measures at Foreign Ports (vessels or aircraft)

   Food and water supply, rodent control and inspection, vaccination, or detention. "Bill of Health" issued to vessel.

   Measures in Transit

   Sanitary inspection and correction, radio report of any communicable disease occurrence aboard.

   Measures at Ports of Entry

   Inspections, examinations, detention, disinfection, fumigation, isolation, surveillance, disinfestation

   Sanitary Inspection - rodent, vermin, insect infestation or other unsanitary conditions requiring measures for prevention of introducing transmission or spread of disease.

"Pratique"

Importation

Lather brushes, psittacine birds, pet cats, dogs or monkeys.

3. Interstate Quarantine Regulations (1946 - Part 12)

Anthrax - lather brushes of hair or bristle manufactured in a certified plant - 250 degrees F for 25 minutes (PHS inspections)

Psittacosis - interstate shipment of psittacine birds is restricte

Trichinosis - heat treatment (boiling 30 min) of garbage prior to interstate shipment for feeding to hogs.

Enteric and Insect Borne Diseases

Water for drinking and culinary purposes potable

1946 DW Standards - inspection of water supplies, certification.

Watering points - piers, railroad coach yards, airports - Plan approval

Land and Air Servicing Areas - inspections - platforms, drainage, watering equipment, bactericidal treatment, ice, disposal of body discharges, garbage disposal.

Vessels - inspections - potable water systems, wash water systems, swimming pools, toilets and lavatories, discharge of wastes, insect control, rodent control

Land and Air Conveyances - plan review-water system, toilet and lavatory facilities, food handling facilities, ice handling equipment, land carrier waste discharge, air carrier waste discharge.

Land, Air, Water Conveyances - FOOD - Source of food,

milk, shellfish

Refrigeration 50 degrees F

Ice - source and handling

Places for preparation, service, or storage of food

Utensils and Equipment

Garbage equipment and disposition

Toilet and lavatory facilities

Health of food handlers

Certificates

Restrictions of Travel of Persons with Communicable Diseases (Permit)

Measures in the event of inadequate local control

4. Shellfish Sanitation Program

Cooperative arrangement between PHS, industry, and State Control Agencies.

State certificates to dealers

PHS - reviews State Control including:

Growing areas, harvesting, shucking plants, purification plants, storage, tagging, etc.

Publishes bi-monthly lists of certified dealers in states where control procedures are acceptable. Lists sent to consuming communities and states.

Interpretation of Stream Pollution Data
(A General Discussion)

(H. W. Streeter)

I. Assumed data

  A. Hydrometric

    1. Discharge (at critical points)

      a. Above and below tributaries, and major sources of pollution

    2. Times of flow (estimated)

      a. Velocity estimates (approx): Velocity - area method; float measurements; NaCl and dye methods

  B. Bacteriological

    1. Plate counts (optional) (24 hrs 37°C)

      a. Useful in measuring effects of tributaries and self-purification

    2. Coliform numbers (M.P.N. or Phelps Index)

    3. Other index organisms (streps, etc)

      a. Not yet accurately quantitative; possible uses in measuring bathing water pollution

  C. Chemical

    1. Dissolved oxygen

    2. B.O.D.

    3. Nitrogen

      a. NH_3

      b. NO_2 & NO_3

    4. Alkalinity (hardness)

D. Physical

1. Temperature, turbidity, threshold odor, etc.

II. Interpretation

A. Hydrometric data

1. Individual discharge estimates

a. Error may reach 20% on individual estimate

2. Averages

a. Error reduced by $\sqrt{u}$, where (u) is number of items in mean

3. Time of flow estimates

a. Error may reach 25 - 30%

b. Estimates worth while, regardless of error

B. Sampling

1. Small samples vs large universe

2. Governed by laws of probability

3. Measured standard error may run 10 - 20% on single dip sampling. Errors random and compensating

C. Bacteriological Data

1. Plate counts

a. Standard error single plating 10 - 15%

b. Probably more accurate than coliform measurements for single determinations

c. Averages - Arithmetic vs. geometric: Frequency distribution curve tends to assume normal form when based on logarithms of counts.

d. Counts are rough index of pollution by sewage and also by unsewered surface drainage water. Self-purification time curves follow same general trend as coliform curves, but somewhat less sharp.

    e. Are a good check on coliform counts especially if only few samples collected.

2. Coliform Numbers

    a. Most specific and delicate indicators of sewage pollution

    b. Also indicate rural and unsewered surface pollution - at low levels

    c. Error of single enumeration may be 15 - 20%, but depends on measured density

    d. Averages - arithmetic mean of M.P.N.'s tends to follow normal frequency distribution more closely than plate counts, or Phelps index

    e. Statistical value of averages (e.g. monthly)
    Error reduced to 1/3 - 1/5 if 9 to 25 samples collected

    f. Variations due to flow (run-off) changes may overshadow errors of sampling and enumeration
    Seasonal variations in per capita contributions: high in summer; low in winter

    g. Good results at critical points in stream will indicate sanitary conditions very closely. Also aid in analysis of relative needs for corrective measures above water intakes. Nearest sources of pollution most critical.

    h. Relation of pollution age to significance of coliform numbers

    i. If average coliform numbers less than 5000, general sanitary conditions in stream usually good (i.e. sewage pollution moderate and D.O. levels satisfactory). If over 20,000 sanitary conditions poor from all standpoints.

D. Chemical Data

1. Dissolved oxygen

   a. Accurate and positive index of stream conditions
   Comparable to coliform numbers as general indicator

   b. Undesirable levels: 0 - 3 ppm
   Doubtful but tolerable: 3 - 5 ppm
   Desirable levels: over 5 ppm

   c. Oxygen "balance" indicators - useful in evaluating temporary stream conditions. Reflect rates of stream recovery

   d. Reaeration - biological and physical
   Relative constancy and importance - depend on stability of conditions

   e. Effect of sludge deposits on D.O. - may exert heavy load in spring from deposits stored in channel during winter

2. B.O.D.

   a. Single determinations by dilution test - errors may be large, but tend to be compensating. Nitrification chief unbalancing factor

   b. Observed reductions in stream

      (1) May be affected by nitrification, overlapping ortransitional

      (2) May be affected by sedimentation and absorption by biological growths in shallow channels

      (3) In summer, approach true index of oxidation with stable flow conditions prevalent for 25 - 30 days. (i.e. in presence of sludge deposits).

c. Evidence that rates of B.O.D. reduction more stable in stream than in lab. test by ordinary dilution method

d. In Ohio River streams, 5 day B.O.D. levels below 3 - 4 ppm usually coincide with good sanitary conditions. Above 4 ppm conditions doubtful or poor. (British standard = 4 ppm). (Not an infallible rule, but a rough guide)

3. Nitrogen

a. Free NH_3 - useful as index of extent of possible chloramine formation in chlorinated water supplies. Also potential source of nitrification.

b. NO_2 & NO_3 - Indicate status of nitrification in stream. In excess, may affect higher aquatic life and also fitness for potable water supplies (cyanosis in infant consumers).

4. Alkalinity, hardness & pH

a. Indicators of dilution changes, effects of acid mineral pollution and ground water infiltration

b. Useful to water works and industries

c. pH has bearing on higher aquatic life

E. Physical data

1. Temperature - essential as record

2. Turbidity - Index of soil wash, sedimentation, scum, etc.

Inventory of Water and Sewage Facilities
Inventory of Needs - Sanitation Facilities
Water and Sewage Facilities Inventory

(R. S. Smith)

HISTORY

In 1939 it was realized that there was a complete lack of anything approximating a descriptive list of sewer systems and sewage treatment plants for the United States as a whole. Various states had published listings of the facilities in their individual states, but for many areas there was nothing available to give information to those who had need of it. Listings had been made in the past for particular areas of the country such as the Ohio River basin and the Association of State Sanitary Engineers had made a general listing early in the 1920s, but these listings had not obtained widespread distribution and were decidedly out of date, having been made before the spurt in sewage plant construction promoted by federal grants and loans. It was realized that there was a definite need for such assembled information and as the Public Health Service has long been intimately connected with sewage treatment and pollution in relation to its effects on the streams of the country, it was felt that the Service was the logical agency to assemble and publish this information.

This work was started in 1940 with a search of the literature for descriptions of all operating sewage treatment plants. From these published descriptions, basic data sheets were prepared giving as complete a summarized description as possible. At the same time, the various State Health Departments were requested (through the Public Health Service Districts) to forward such information as was available on the sewer systems and treatment plants under their jurisdiction. Some of the states had typed or printed lists of the systems in their state, but generally the descriptions were not as complete as was desired. One or two states had the information on file cards, but were unable to assign the necessary personnel to make copies of the cards to furnish them to us. This difficulty was overcome by having an engineer from this station go to the state capitol and photograph all the file cards. Enlarged prints were made from these negatives and became a part of the basic data file of the inventory. In some cases, the desired information could only be obtained by abstracting from typed reports of inspections of the plants made by State Health Department personnel. As these data were collected from various sources, tabular forms to record the important information and symbols to describe the treatment processes were developed. It was believed that the value of the census would be considerably increased if the drainage basins in which sewer systems were located were indicated. In order to have a drainage basin arrangement that would be applicable to the country as a whole, the drainage basin arrangement established by the National Resources Committee was used. These drainage basins are outlined in "Drainage Basin Problems and Programs," National Resources Committee, December, 1936. During the years that we have been working on these inventories, this drainage basin scheme has been found to have certain disadvantages due to the fact that the areas delineated are not true drainage basins, but rather economic areas based on the river systems. Some very incongruous situations have consequently arisen. For instance, Quincy, Illinois is on the Mississippi River approximately 100 river miles above the mouth of the Illinois River, but it is listed as in the Illinois Basin. Then there is the St. Louis Basin which has no connection with a St. Louis River, but is an area on both sides of the Mississippi River around St. Louis, Mo., and East St. Louis, Illinois. We are now redrawing these basin boundaries as rapidly as we can to make them conform to true drainage basins, subdividing certain basins that contained several completely independent river systems, and rearranging the identifying symbols so that any given basin will always be identified by the same symbol and that the same symbol will not be used for more than one basin.

The information gathered from all sources was typed on tabulation forms for each state (the communities within the state being arranged alphabetically) and sent to the State Health Department for checking. After the forms were returned with any necessary corrections, stencils were cut, the material mimeographed and bound into sections by states. A number of volumes were made up to form a complete inventory for the United States for distribution where such a complete inventory was needed.

Upon completion of this inventory of sewage facilities, a similar inventory of water treatment plants was completed in 1941. Annual revisions for these inventories were made to show additional systems and major revisions to existing facilities for the years 1941, 1942 and 1943.

In 1944 it was decided that to complete the inventory of water and sewage facilities, a comparable listing of untreated water supplies was required. Again the State Health Departments were requested to furnish all available data from the files, another set of tabulation forms was devised, the data assembled and tabulated, checked, stencils cut, the material mimeographed, bound into volumes and distributed. This inventory of untreated water supplies was completed in January 1947 and was essentially as of December, 1943.

Present Combined Inventory

Before the untreated water inventory was completed, it was realized that there was a great need for a combined inventory that would show the water and sewage facilities for a given community on a single sheet. A sheet 16 x 19½ was required to tabulate properly the information with a typewriter. This sheet size is so large that we do not have the equipment to cut stencils and mimeograph the material. And, even if it were possible to mimeograph this sheet, it would be so large that it would be very awkward to handle and almost impossible to file. The complete inventory will consist of approximately 900 pages and this would make an excessively thick volume. For these reasons it was planned that this first combined inventory (which is as of December 1945) would be set up in type and printed for distribution and that it would be printed every five years thereafter. Corrections were to be made annually and corrected sheets retyped, but these interim inventories would not be for general distribution, a single copy for each state being furnished to the Health Department of that state for file record. Due to lack of funds in the printing appropriation of the Service, however, it will be impossible to have this inventory set in type. It is expected that it will be possible to reproduce it by photo-offset printing when it is completed this spring.

(TYPICAL SHEET AND MIMEOGRAPHED "NOTES ON TABULATION"
TO BE DISTRIBUTED)

Future Plans

Annual Revisions

It is planned to send a carbon or blackline print copy of the annual inventory to the State Health Department in order that any entries for additional systems or plants may be entered and any changes made in the existing entries due to additions or revisions of plants, additional or different sources of supply, or later information on populations served or average outputs. Before these copies are sent out for correction, it is planned that this office will enter any information that we have been able to find in such a manner that the correction will be easily noticed. If we have found references in the literature or in Engineering News-Record's Construction news of new construction that we think may affect the inventory entry, we will note it in an accompanying letter. It is anticipated that this method will enable the State Department to make a complete entry with a full symbolized description of the plant in question. There are many items of information that are missing, particularly dates of original systems, date of present water source, date of start of operation of present plants, estimated populations served, and adequate descriptions of plants. It is hoped that this information can be gradually obtained by the various Health Departments and will be added as it becomes available.

When these corrected sheets are returned to this office we will go over them, if any apparent discrepencies or errors are found (possibly water consumptions of 300-700 g.c.d. or consumptions under 10 g.c.d. as have at times been reported), send letters of inquiry concerning such questions to the state, and then finally retype such sheets as have been changed. A complete copy of the corrected inventory for the one state will then be sent to the State Health Department for their records.

It is hoped to have the complete inventory reproduced for general distribution every five years.

To maintain this program of annual revisions and the publication of the complete inventory every five years will require wholehearted cooperation by the State Health Departments. If we can complete the 1947 revision by December, 1948, we will be able to send out copies for 1948 revisions in January, 1949. If the corrections are promptly made and the copy returned, it is hoped that the 1948 revision will be completed by December, 1949. In this connection, it is important to note that it requires over 300 man-days to type and proof-read the approximately 900 pages of the complete inventory or approximately 1½ man-years. There are two clerks and an engineer assigned to the Inventory work, which makes it practically impossible to do more than barely keep abreast of the annual revisions.

Basic Data File

It is planned to have a basic data file containing all information that can be assembled descriptive of the water and sewage facilities of each community in the United States. This will require a folder for each community listed in the inventory. These community folders will be arranged alphabetically by states. Into these folders will be put all material that we can gather concerning the facilities of that community, such as descriptive articles from the technical literature, pictures from advertisements in the engineering magazines, annual reports (if available), typed description of plants and treatment units, etc. Considerable progress has been made on this project, but it is far from complete. We have on hand files of Water Works Engineering, Sewage Works Engineering, Public Works, and Water and Sewage Works from about 1942 to date from which we plan to clip the material mentioned for this basic data file, but the personnel for this work has not been available. If this file can be completed as planned it will provide much more complete information on the past history and present condition of the facilities of any community than can be given by the inventory entry.

Punch Card System

It has been planned to enter the information from the inventory on coded punch cards to facilitate statistical summarization of data. Coded punch cards for both water and sewage facilities were designed and purchased, but personnel has not been available to code and enter the information. Some coding of information was done on an experimental basis and detailed coding instructions were written for both the water and sewage cards. From the experience gained in this trial coding, it is estimated that it will require a year's time of one engineer merely to mark the cards for punching and to enter the most essential descriptive information that could not be coded.

(Sample code cards to be distributed)

McBee Key-Sort cards were selected for this purpose for two reasons. One major reason was that the equipment for this system is much lower in cost for the IBM system and it is not necessary to rent expensive sorting equipment. Another advantage is that the Key-Sort card allows for the entry of considerable information that is not particularly suited to code punching.

Advantages to State of Inventory

Many State Health Departments have in the past issued lists of water and sewage facilities in one form or another. Other states have apparently not found their records of facilities in such condition that the information could be readily tabulated into lists and have not had the available personnel to do this work. This cooperative inventory will provide a listing for each state annually that could be reproduced for wide spread distribution within the state and at a minimum of expense.

The annually revised inventory will provide a convenient descriptive listing for the State Health Department of the water and sewage facilities for each community in the state and show those communities that are without such community facilities. This will enable the department to plan its work to the best advantage and indicate where promotional work would be likely to be most fruitful.

Inventory of Needs - Sanitation Facilities

During the years 1945 and 1946 a survey of needs of water, sewerage and garbage was made covering the entire United States. Health departments of the various states, as well as hundreds of county and city health departments cooperated with the Public Health Service in making this the most complete inventory of basic sanitation needs ever compiled for the nation. In each state a random sample of communities in each population group was selected and engineers were sent to each of the communities selected to obtain information concerning the existing facilities and estimated needs and their cost. Information was obtained from superintendents of the water and sewer systems, mayors, other community officials and leading citizens, as well as from county and State Health Departments. This information was entered on individual sheets for each community surveyed. The accuracy of the estimate of construction needed and the estimate of cost naturally depended upon the source of the information and how far local planning had been carried.

This information on type, amount and cost of construction was extrapolated from the sample to the population group and then summarized for the state. The detailed report on this survey has not yet been published, but a preliminary release made last July shows that on the basis of June, 1946 costs, the nation needs 2.2 billion dollars worth of water works construction to serve more than 81 million persons in cities and and towns, that the sewage facilities needed by 85 million persons in communities over 200 population would cost approximately 3.7 billion dollars. In towns of less than 1,000 population, the costs of needed facilities exceed an average of $100 per capita, and in cities of over 100,000 the costs are over $80 per capita. In communities of intermediate size, the per capita cost is about $50.

Investigation of Industrial Wastes Problems

(R. C. Palange)

1. Introduction - Part of Broad Pollution Survey
    - a. Purpose - To determine volumes, and locations, and characteristics of wastes.
      To aid in deciding on remedial measures.
    - b. Scope - All parts of plant, whether or not suspected of production of polluting wastes.

2. Public Relations
    - a. Preliminary Contacts - with high officials
    - b. Cooperation by industry - collect samples, run analyses in parallel, use results.
    - c. Hold data from industry confidential.

3. Organization
    - a. Staff - should include:

      Engineers to handle hydraulics, interpretation of data, reports, tie project together.

      Chemists - to handle laboratory work.

      Bacteriologists - often not needed except where water supply problems directly involved.

4. Collection of Preliminary Data
    - a. Description of Process - Necessary for determining sources of wastes and possible control measures.
    - b. Raw materials and products - Permits comparison with other plants - can estimate quantity of wastes.
    - c. Sources of Wastes - Aids in setting up flow measuring and sampling program.
    - d. Quality of Wastes - Obtain available analyses of other similar plants for setting up laboratory determinations.
    - e. Recovery Practices.
    - f. Waste treatment other than recovery - Determine function and efficiency of treatment units.
    - g. Sewer Plants - Aids in locating sampling and gaging points.
    - h. Disposal of sanitary sewage - Often separated and goes to City sewers.
    - i. Source of Water Supply - Determine quality of intake water and subtract from total plant load.

5. Sources of Assistance

   a. State and Municipal Agencies - Legal aspects, sewer ordnances, effluent requirements, treatment processes.

   b. Federal Agencies - U S G S (hydraulics), Corps of Engineers (navigation pollution laws), U S P H S (laboratory procedures, guides), Fish and Wild Life Service.

6. Location of Sampling Points

   a. Final sewers - at or near entrance to stream.

   b. Intermediate sewers - to determine principal sources of concentrated wastes and to check results on final effluent.

   c. Special points in plant - Centrifuges, concentrators, Screens, etc.

7. Sampling Program

   a. Necessary to calculate time required of personnel to:

      (1) Travel between sampling points.

      (2) Make necessary hydraulic measurements.

      (3) Collect individual and composite samples.

      (4) Carry out the required laboratory determinations.

   b. Frequency

      (1) Limited by personnel in both field and laboratory.

      (2) Should be determined by variations predicted from the operation of the waste producing process.

         a. In case of individual machine with steady operation - only few hours may be necessary to rate wastes to production.

         b. In case of outfall sewer with multiplicity of wastes - minimum of 24 hours and up to 5 days required. Judgment necessary in each individual case.

         c. Seasonal consideration - canning, etc.

   c. Use of composites

      (1) Samples should be taken proportional to flow.

      (2) Valuable for taking large numbers of samples of highly variable waste flow without imposing too great a burden on the laboratory.

(3) Solids determinations, other inorganic or relatively stable organic compounds, or other compounds which require lengthy laboratory procedures especially adaptable to composite sampling.

(4) Little value to biologist on determination of minimum lethal dose to fish as extremes are the important factor.

8. Hydraulic Measurements

a. Types of Outlets:

(1) Circular sewers.

(2) Oval shaped sewers.

(3) Rectangular Flumes.

b. Discharge conditions:

a. Underwater discharge

b. Atmospheric discharge

c. Survey of possible means of Measuring Flow:

(1) Conditions to ascertain

(a) Volume of flow expected.

(b) Type of material in flow.

(c) Access to flow.

d. Methods available

(1) Weirs.

(2) Current Meter.

(3) Pitot tube.

(4) Chemical methods.

(5) Miscellaneous - Water Meters, Pump Ratings, etc.

e. Weirs

(1) Accuracy of selected type and size.

(a) Calculation of weir size required by assuming expected flow.

(b) Availability of locating weir.

(2) Possible head measuring devices

(a) Float level recorder - best because it gives continuous record.

(b) Hook gauge

(c) Graduated rod

(3) Types

(a) Triangular

(b) Rectangular

(c) Trapezoidal

f. Current Meters - for large sewers or channel

(1) Access to flow

(a) Bridges

(b) Cable suspension

(c) Manholes

(2) Preliminary survey of chosen Measuring Section

(a) Cross-section of flow

(b) Divide depth of flow to .2, .6, and .8 depths.

(c) Current meter readings at numerous intervals across flow.

(d) Calculation and averaging of all current meter readings.

(e) Current meter readings over a long period of time to develop stage - discharge curve.

(f) Pitot Tubes:

(1) Methods

(2) Limitations

(a) Solids interference

(b) Turbulence

(g) Chemical Methods:

(1) Accuracy - Dependent upon chemical analysis and feeding mechanism

(2) Adaptability

(a) Chlorides for low flows

(b) Sulfates for higher flows

(h) Floats:

(1) Small use except on Streams

(2) Dependent upon accurate measurement of water cross-section

(3) Straight section of stream required and no interfering flows

(i) Miscellaneous:

(1) Pump operation and water meter records

(2) Use of dyes

9. Laboratory Program

a. Consultation with laboratories prior to survey

(1) Establish actual location of sampling points

(2) Determine specific analyses required

(3) Agree on number of samples and size

(4) Arrange for definite transportation

(5) Set up schedule for reporting results

(6) Carry on the necessary preliminary explatory tests to determine at least qualitatively the compounds expected in the effluent.

b. Choice of Analytical Procedure

(1) Literature replete with short cuts and modifications - should be avoided.

(2) Sanitary field has two "Bibles"

a. Standard Methods for the Examination of Water and Sewage, American Public Health Assoc., 1790 Broadway, New York City 19, N. Y.

b. Laboratory Manual for Chemical and Bacterial Analysis of Water & Sewage by Theroux, Eldridge and Mallman, McGraw-Hill. (Technical Periodicals-Ind. & Eng. Chem.-Sewage Works Journal, etc.).

(3) Strict adherence to Standard Methods recommended in case of possible litigation, etc.

(4) New techniques now developing in field of colorimetry, photelometers, electro-photometer.

(5) Use of private laboratories for special, but very informative tests, X-ray diffraction, spectroscopic, etc.

c. Sample handling and storage

(1) Attempt to influence rapid delivery to laboratory.

(2) Agitation and consequent aeration must be kept at a minimum.

(3) Preservation of samples

a. Analyze immediately if possible.

b. Icing best general method (40°F).

c. Formaldehyde, although formerly in common use as a general preservative, no longer recommended.

d. Impossible to preserve samples for D. O., B. O. D., and bacteria.

e. At room temperatures B.O.D. may be lowered 10 to 40% in six hours.

f. Chloroform 5 ml. per liter usually satisfactory as general preservative.

g. H_2SO_4 at dose of 1500 p.p.m. will maintain nitrogen balance.

h. $CuSO_4$ for phenols, 10 ml. 10% solution per liter of sample.

d. Required Equipment

(1) See lists in laboratory manuals

(2) Consult any of the agencies previously listed or local sewage or water plant.

(3) Minimum Equipment Required

(a) Balance sensitive to 0.01 gram
(b) Nessler tubes and rack
(c) Drying oven
(d) Water aspirator
(e) Beakers
(f) 8 oz. glass stoppered bottles
(g) Burettes
(h) Bunsen burners
(i) Corks
(j) Gooch crucibles and holders
(k) Suction flasks
(l) Erlenmeyer flasks
(m) Evaporating dishes - porcelain
(n) Filter paper
(o) Funnels
(p) Graduated cylinders
(q) Volumetric flasks

10. Interpretation of Results of Survey

a. Expression of results

(1) Parts per million
(2) Pounds per million gallons
(3) Grains per gallon
(4) Quantity units

a. Pounds per twenty-four hours
b. Population equivalents

b. Determine if pollution problem exists - Consider concentration of wastes rather than pounds.

c. Compare findings with Stream Standards, Guides, or Sewer Usage Regulations.

d. Calculate results of studies of quantities of wastes to production units.

e. Make a careful search of literature before accepting or suggesting any lethal limits of wastes in receiving water. (There is a wide variation of opinion on "what is considered too much").

11. Preparation of Report:

    a. Address to layman and technical man.

    b. Include material under "Collection of Preliminary Data."

    c. Describe all procedures in detail.

    d. Summarize laboratory and hydraulic data.

    e. Differentiate between facts and items based on individual estimates or judgment.

12. Engineering Investigations

    a. Depends on existing data.

    b. Does not involve sampling and gaging program.

    c. Reports should carry essentially same information as reports on sampling studies.

Rural Sewage Disposal

(S. R. Weibel, J. R. Thoman, C. P. Straub)

I. General

Through arrangements made in late 1946 between the Public Health Service and the Housing and Home Finance Agency, a Household Sewage Disposal Project, sponsored by H.H.F.A., was set up in the Health Service, to be conducted by the Water and Sanitation Investigations Station at Cincinnati.

Although the Project materialized from difficulties encountered by the National Housing Agencies, investigation of some 600 sources of information indicate conflicting ideas and general confusion because of lack of a solid foundation and the perpetuation of various opinions; moreover, that the problem is not only country wide, but world wide. Some 17 million persons in this country depend on individual household septic tank soil absorption systems. Past attention given to factual study in this important field has considerably lagged that given to municipal treatment (activated sludge and trickling filters, alone or with other processes, serve 11 and 8 million persons, respectively, in this country).

The list of study topics set up for the Project include: a review of literature; inspection of existing installations; a program of research, including experimental studies of septic tanks, subsurface systems and effluent soil clogging characteristics; sampling and gauging, the feasibility of providing for longer intervals between necessary cleaning; study of the effect of extreme freezing conditions, of tree and shrubbery roots on absorption, of residential garbage grinding on septic tank operation; study of distribution boxes; tank shape, etc., to permit shorter detention and larger sludge storage capacity; the effects of grease; of sodium and calcium to soil clogging; of seepage, evaporation and transpiration to overall efficiency; the study of new methods; and, finally, the development of recommended standards of design and operation.

A review of sources of information was started in January, 1947 has covered some 600 references thus far. Most of these deal with design. Less than 10 factual studies were unearthed, together with two current studies under the sponsorship of the U. S. Public Health Service Grants-in-Aid Program.

Inspection of individual systems in the field, begun in Dallas in February, 1947, have been extended to other areas. Inspections are being made on the uniform basis of a standard form, involving the measurement of tank, scum and sludge, inspection of absorption field, which, with statistics on household, water supply and history of system, complete a report.

The experimental research program got under way in June, 1947, with operation of 8 full sized septic tanks, subsequently increased to 16, representing a variety of tank designs, all operating in parallel. Table 1 presents the data pertinent to each tank.

(Table given on following page)

Tank No.	Total Liq. Cap.	Description			Liq. Depth	Remarks
		W.	L.	Dia.		
1.	2060	5'-0"	11'-0"	-	5'-0"	Rect.Jt.Cpm. type
2.	513	2'-11"	5'-10"	-	4'-5"	Roundedends
3.	553	-	-	2 at 3'-0"	5'-3"	2-comp.Vert.Cyl.
4.	673	-	-	4 at 2'-0"	7'-3"	4-comp.Vert.cyl.
5.	525 Max	3'-4"	6'-0"	-	4'-3"	Oval
6.	455	-	-	4'-4"	4'-0"	Single vert. cyl.
7.	539	3'-0"	6'-0"	-	4'-0"	Rect.Jr.Com. type
8.	492	-	5'-0"	4'-4"	3'-9"	Hor. cyl.
9.*	539	3'-0"	6'-0"	-	4'-0"	Duplicate #7
12.	123	-	-	2'-0"	5'-3"	Single vert.cyl.
13.	246	-	-	2 at 2'-0"	5'-3"	2-comp. vert.cyl.
14.	289	-	6'-6'-3'	2'-0"	1'-6"	3-comp.hor.cyl.
15.	539	3'-0"	6'-0"	-	4'-0"	Dupl. #7
16.	539	3'-0"	3'-3'	-	4'-0"	2-comp.rect.
17.	539	3'-0"	2'-2'-2'	-	4'-0"	3-comp. rect.
18.	405	3'-0"	6'-0"	-	3'-0"	Rect.

*Receives ground garbage in addition to sewage.

Eleven of the tank installations approximated 500 gallons each in capacity; one, 2,000 gallons; and the remainder, less than 500 gallons each. These batteries were operated on a simulated household flow pattern. Testing was accelerated by adding amounts of sludge, obtained in the field, to the tanks at intervals, before the start of each run. After seeding and preliminary operation of 8 of the tanks at 500 g.p.d. rate, the entire battery was dosed at 250 g.p.d. per tank over a range of sludge accumulations. Routine analyses on composited samples included temperature, pH, and solids. Special examinations included B.O.D., sulfides, grease, and bacteriological determinations. Other work undertaken embraces study of the effects of tank seeding, zeolite softener salts, dosing through sludge, the bacteriological aspects of clogging. Six 50 ft. experimental absorption lines are being investigated. Field sampling and gauging of operating installations were started.

Other activities include circularization of local health departments having a full time health officer, concerning control, difficulties, servicing, and costs; and northern plumbers, concerning the freezing aspects of shallow systems.

On conclusion of the study, or parts of it progressing to a stage sufficient to warrant definite conclusions, the information will be made available to all interested, through Government publication.

II. Review of literature

A. General

1. Scope - over 650 references from all over world

2. Methods - various Indexes - used Union List of Serials to locate libraries having articles

3. Material - factual data scarce

B. Historical

1. Mouras - Vesoul, France in late 1860's

2. Moigno - experiments with Mouras and patenting of tank by Mouras in 1881

3. Philbrick - first household septic tank in U. S. in 1883

4. Cameron - patented septic process in 1895 in England - later in U. S.

C. Research Programs

1. University of Illinois - 1920's - Lehmann, Kelleher and Buswell

2. Kansas State College - 1920's - Walker and Driftmeir

3. Australian study - 1930's - survey of operating tanks

4. Others - University of Minnesota - Iowa State College- Ontario Agricultural College

5. Present studies

a. University of Michigan - septic tanks

b. Rensselaer Polytechnic Institute - absorption by soil of effluent

c. University of Massachusetts - completed - to be published soon

D. Practice

1. General - world wide information gathered

2. United States - State's recommendations tabulated

3. Germany - fresh water clarification tanks - small Imhoff tanks

III. Field Surveys

A. Standard inspection form

1. Procedure

a. Permission

b. History

c. Uncovering tank

d. Tile field exam each 5th survey

2. Criteria

a. Adequate history exists

b. Outlet elbow, tee or baffle intact

c. Tank not completely full of scum and sludge

B. Analysis of data

1. Rate of scum and sludge accumulation

2. Case history on certain items

C. Percolative tests

1. Ryon's formulation of test

2. Modified test - 10 in. dia. tube

3. Field tests

a. In tile field trench

b. Outside tile field

c. Soil samples taken at those points - mechanical and hydrometer analyses - attempted correlation with percolation test results

Sanitary Surveys and Reporting

(D. J. Schliessmann)

I. Introduction

- A. Title is all inclusive of activities as entered into by a health department
    - 1. Examples of types of surveys
        - a. Water, milk, food handling establishments, industrial hygiene, malaria, typhus, housing, rural surveys, stream pollution, industrial wastes, etc.
- B. Surveys or programs discussed fully later in present course
    - 1. Stream
    - 2. Industrial wastes
    - 3. Water and sewage
    - 4. Milk and food
- C. Broad usage of term "sanitary survey," and its application does not permit establishment of a definite procedure for surveys.
    - 1. Definition
        - a. Sanitary - "occupied with measures or equipment for improving conditions that influence health."
        - b. Survey - "a critical examination or inspection, often of an official character, for an implied or specified purpose."
        - c. Sanitary Survey - "a critical examination or inspection of conditions that influence health for the specific purpose of improving unfavorable conditions."
    - 2. Importance of definition in entirety because it aids in our obtaining a complete picture of the overall principles of a problem followed by the formulation of plans to accomplish our results.

II. Methods of arriving at problem by asking ourselves the five "W's."

- A. Why
- B. What
- C. Who
- D. Where
- E. When

III. Significance of five "W's."

A. Why

1. Why is the survey important?

   a. Public Health implications
   b. Economic reasons
   c. Necessity of providing direct service to the people
   d. Political

B. What

1. What is the objective and what is to be accomplished?

   a. In the furtherance of public health
   b. In economic returns
   c. In the development of good will and future support

2. What data should be collected

3. What methods are available for

   a. Making of survey
   b. Obtaining corrective measures

4. What budget will be required

5. What personnel will be required

6. What will be the immediate and later reaction of the populace?

7. What direct services to the populace can be rendered?

8. What cooperating agencies can be called upon

C. Who

1. Who will administer the program
2. Who will finance the program
3. Who will do the survey
4. Who will do the corrective work
5. Who will benefit the most

D. Where

1. Consideration of point where survey is to begin and where operations will be inaugurated.

E. When

1. When will survey operations and/or control operations begin.

IV. Direct Service Programs for the People

A. Health work stimulated during the war and scope of sanitation activities increased.

B. Basic consideration for continued expansion of Health Departments.

1. Provision of a service to the people that no other agency can render which will provide tangible results.

2. Trend is toward establishment of service programs for the benefit of the people rather than consultation programs.

C. Programs that Health Departments can inaugurate that are self supporting and will obtain public support and approval.

1. Garbage collection and disposal programs
2. Rodent control programs
3. Insect control programs
4. Septic tank programs
5. Well drilling programs
6. Pit privy programs
7. Cooperation with city engineers and real estate developments for provision of water and sewer facilities to outlying areas.

Sewage Treatment Plant Control Tests and Operating Problems
(J. R. Thoman)

I. Control Tests

  A. State health dept. lists of tests

    1. Various types and sizes of plants

    2. To fit individual needs and circumstances

  B. Small plants

    1. Use as many tests as possible

    2. Require at least relative stability in all cases

II. Operation

  A. Introduction

    1. Much data available concerning process control and maintenance.

    2. Little information concerning design for operation

  B. Overall specifications

    1. Sell city on operation when selling plant

    2. Provide landscaping in contract

    3. Specify that equipment manufacturer place his equipment in operation.

    4. Insure that complete survey of water usage and various wastes has been made.

  C. General points

    1. Provide metering

      a. At least for total flow

      b. At other points consistent with process control

      c. Use indicator at meter

      d. Place recorder in control house.

2. Provide adequate stop gates to provide flexibility and elininate dead ends.

3. Provide bypasses to separate plant units into constituient processes and around entire plant.

4. Screen all windows.

5. Provide locker room, toilet, and shower.

6. Provide an office, desk, and file.

7. Adequate water supply in lab, at primary tank, and other strategic points around plant.

8. Use stairways instead of ladders.

9. Tie tanks down to prevent floating.

10. Provide adequate ventilation in chemical storage rooms, digester control rooms, pump pits, etc.

11. Night lighting

12. Plugs in tees for rodding lines.

D. Screens and Grit Chambers

1. Provide mechanical cutting of solids wherever possible.

2. Bypass screen and drain platform for screenings.

3. Avoid manually cleaned screens in deep pits - (pump pits) apt to be neglected.

4. Provide drainage of screen and comminutor channels when not in use.

5. Sufficient stop gates to allow multiple or single use of facilities.

6. Have no dead spaces for settling of solids.

E. Primary tanks

1. Even distribution to units and even distribution within a unit.

2. Adequate baffling - keep at least 1 inch below w.s.

3. Scum removal device - and provision for disposition of scum preferably to digester - eliminate can disposal or pits.

4. Eliminate flat undersides where sludge and scum can collect.

5. In hoppers of rectangular tanks with sludge collector provide a 60° minimum slope - have adequate sludge storage, so that it does not have to be withdrawn too often-if more than one hopper provide gate valve to control discharge from each hopper.

6. Imhoff tanks - provide for squeegeeing of sides of flowing - thru chamber - provide steps or chains for getting out where tank top is high above water level.

7. Have some workable means of observing condition of primary sludge - periodic withdrawal of sludge is necessary in small plants - sludge well and draw-off pipe can be used to advantage.

F. Secondary Treatment

A. Sand filter - use correct size and graded sand - provide for draining of dosing tank - in case of shut down, and to remove settled sludge.

B. Trickling filters - provide for flooding filters

1. Dosing tanks of such size that rest periods do not exceed one hour.

2. Provide drainage of dosing tanks.

3. If no multiple units then arrangement to confine flow to portions of filter.

4. Fine screens to remove nozzle clogging material - provide for washing of screens.

C. Activated sludge - mix primary eff. and return sludge before the mixed liquor enters aeration units. Necessary where division to multiple units is made.

1. Sludge division box of sufficient size to take design return plus at least 100% - or to take pump capacity.

2. Well designed flow dividing device - adjustable.

3. In larger plants provide mutiple introduction of return sludge or primary effluent.

4. Final settling - workable continuous sludge withdrawal devices.

5. Minimum slope hoppers.

6. Adequate weir length no flat undersides for collection of sludge.

7. Some comments given under primary tanks are applicable

G. Sludge Digestion Tanks

1. A convenient method should be installed for observing the supernatant and for withdrawing from several depths in the tank.

2. Keep piping as short as possible in and around the digester.

3. Keep valves outside of a digestion tank.

4. Provide suitable arrangements for measuring the tank temperature.

5. Digesters should always be insulated.

6. Install danger signs around tank - include in original contract.

H. Sludge Beds

1. Large beds should be provided with driveways to facilitate removal of sludge.

2. Keeps walls as low as possible to expose all the surface to the sun.

3. Keep the slope toward the drains, and return the drainage through the plant if at all possible.

I. Pumps and Piping

1. Keep machinery indoors.

2. Eliminate outside valves.

    a. corrosion
    b. freezing
    c. tendency not to use in bad weather
    d. usually lost

3. Keep motors off floors to avoid damage by washings and floodings.

4. Use pumps to fit the service intended.

plunger and scru-peller - primary sludge
centrifugal - recirculation and act. sludge
eliminate submerged suction pumps

5. Provide duplicate pumps in all cases.

6. Have electrical starting equipment furnished by mfg. of pumps.

7. Use automatic float switches on overflow or drainage pumps.

8. Keep piping as short as possible - yet allow room to work around pumps - room to operate valves.

9. Provide large size sludge lines.

10. Keep valves close to units - prevent sludge accumulation.

11. Check piping arrangements to see that arrangements are OK for

    a. Normal plant operation

    b. Extraordinary plant operation

    c. Dewatering of units.

    Flexibility is important. Valves in ititial cost are much cheaper than emergency service.

12. Provide for dewatering of all units to plant influent or plant bypass.

13. Provide for recirculation of digester contents.

14. Provide return of digester supernatant to plant influent or sand beds.

15. Provide return of waste activated sludge to primaries or to concentration tanks.

Water Quality Standards

(F. E. DeMartini)

1. INTRODUCTION

a. Reference to paper by George Schroepfer on "An Analysis of Stream Pollution and Stream Standards," (Sewage Works Journal, Sept. 1942, pp. 1030-1063).

b. Need for careful consideration of local conditions of water use and necessity in developing a standard rather than adoption of a single standard on a wide geographical base.

c. Stream uses govern requirements or standards and will differ according to these uses. "The key problem in planning for pollution abatement is to find the standards in each section of a stream which express the best balance between the stream's use for receiving and assimilating sewage and other waste and its use for other purposes, aesthetic and economic." (Third Report of Special Advisory Committee on Water Pollution of the National Resources Committee).

d. Tendency in setting up standards or classifications has been to utilize laboratory measures of stream pollution as the coliform group of bacteria, 5 day B.O.D., D.O. Also used are freedom from sludge deposits, floating solids, slick, and odors. Turbidity, alkalinity or acidity and pH value may frequently have special importance.

2. DISCUSSION OF WATER USES AND FACTORS CONTROLLING SUITABILITY OF WATERS FOR THESE USES

a. Domestic water supply

1. Bacteriological quality of raw water - Group I, II, III, IV, see Manual of Recommended Water Sanitation Practice - Public Health Bulletin No. 296, U.S.P.H.S.
2. Other raw water requirements - Freedom from toxic, taste-producing or otherwise harmful substances, or organisms not readily removable by ordinary water treatment processes. Free of excessive amounts of acid. Minimum alkalinity of 15-20 ppm. desirable.

b. Bathing Waters

1. Variability of existing standards and lack of well established observational data to support them.
2. Reference to two standards based on relation between observed quality of bathing waters and sanitary conditions affecting such waters as determined by physical surveys. (Bacterial Pollution of Bathing Beach Waters in New Haven Harbor. CEA. Winslow & D. Moxon, Americal Journal of Hygiene 8, 3, 299-310, May 1928) (Design, Equipment and Operation of Swimming Pools and other Public Bathing Places, APHA, 1942)
3. Need for basic work, particularly epidemiological studies for firm establishment of standards for bathing waters.
4. In addition to satisfactory sanitary quality bathing waters should not be toxic or irritating and should be free from oils, tars, greases, floating solids, scum or debris and low in color and turbidity.

c. Maintenance of Fish Life

1. Limiting concentrations of dissolved oxygen and permissible pH range. (Reference Bulletin No. 22, U.S. Bureau of Fisheries 1937 - by M. M. Ellis, Detection and Measurement of Stream Pollution)

2. Dissolved oxygen

Minimum Daily Avg. D.O. in ppm	Stream Condition
0-3	Heavy pollution, probably nuisance at times, little fish life
3-5	Moderate to heavy pollution, no nuisance, fish life restricted to coarse species
over 5	Slight to moderate pollution, fish life varied, abundant and health, game fish at higher minimum levels

3. Summary of pH variations and effects

Avg. daily values	Stream Condition
6.5 - 8.6	Normal for unpolluted streams
5.5 - 6.5	Moderate acid pollution, tolerable to fish life
4.0 - 5.5	Moderately heavy acid pollution detrimental to fish life
less than 4.0	Heavy acid pollution, lethal to fish life

d. Irrigation Waters

1. Colorado, California and Oregon regulations concerning irrigation with sewage-laden water and sewage effluents.

Colorado - Requires written permission of State Board of Health for use of sewage or sewage polluted waters when Coli-aerogenes group exceeds 10 per cc.

California and Oregon - Regulations similar - Prohibit use of raw sewage for irrigating growing crops. Prohibit use of settled or undisinfected sewage effluents on growing vegetables and garden truck. Highly oxidized and disinfected sewage effluents can be used provided they meet drinking water quality in Oregon and in California not over 10% of 10cc portions are positive for the Coli-aerogenes group. Also no single sample can be positive in over half the 0.1 cc portions examined.

2. Need for accumulation of epidemiological evidence, research on index organisms, and viability of organisms under conditions of crop irrigation and handling from farm to consumer for development of sound standards for irrigation waters.

c. Water Quality for Shellfish Growing Areas

1. Review of water quality provisions of P.H. Bulletin No. 295 - U.S.P.H.S. Manual of Recommended Practice for Sanitary Control of the Shellfish Industry - 1946. Coliform limits for various growing areas are:

Approved Areas - Coliform group organisms not to exceed median content of 70 per 100 ml, expressed in MPN, in a series of samples from each station.

Moderately Polluted Restricted Areas - Median bacteriological content lies between 70 and 700 per

100 ml.

Grossly Polluted - Closed areas - median bacteriological content exceeds 700 per 100 ml.

f. Industrial Water Uses

1. Review of paper by Sheppard T. Powell - "Some Aspects of the Requirements of Water for Industrial Use," Journal AWWA - January 1948. Discussion of water quality requirements for use of water by industry for cooling purposes, processing, boiler feed, air conditioning, sanitary, fire protection and miscellaneous purposes.
2. Review of tabulation of "Quality Tolerance of Water for Industrial Use" - Progress report of committee of New England Water Works Association (Journal of N.E.W.W.A. 54:261, 1940)
3. Reference to material presented by R. F. Goudey in paper entitled: "Developing Standards for the Protection of Ground Water." (Journal A.W.W.A. Oct. 1947)

3. REVIEW OF STATE REQUIREMENTS AND STREAM STANDARDS

a. Summary of current status in states based on recent circularization of questionnaire to the states. Ten states have some type of water quality requirements or effluent or stream standards. Thirty two states have no standards. Three states are currently developing water quality requirements. Three states did not reply.

b. Opinions on usefulness and practicability of application of stream standards among the states vary from those definitely opposed to them to those, of course, which already have them. In general standards or water quality requirements are regarded to be useful as guides and for classification purposes rather than as inflexible requirements to be generally and strictly enforced.

4. REVIEW OF CERTAIN WATER QUALITY REQUIREMENTS NOW IN EFFECT OR PROPOSED

a. Specifications for the Classification of West Virginia Streams - (from Kanawha Basin Zoning Report - West Virginia Water Commission July 1, 1947)

b. Minimum Acceptable Qualities of Water for Various Uses, Proposed for Surface Water in Tennessee (From report: Stream Pollution in Tennessee 1943 - 1944)

c. Minimum Acceptable Qualities of Water for Various Uses in the Tennessee Valley (From Studies of the Pollution of the Tennessee River System, T.V.A., 1945)

d. Ohio Basin - Water Quality Requirements - Summary of Limiting Quality Requirements for Stream Waters with Principal Stream Uses and Conditions Involved in Each Category. (From report on Ohio River Pollution Control, Part II, U.S.P.H.S. 1944, House Document No. 266, 78th Congress)

e. Minimum Water Quality Criteria for Streams in the Potomac River Basin (From fifth annual report - Potomac River Basin Commission)

Industrial Waste Guides

(S. R. Weibel)

As part of the industrial waste program in connection with the Ohio River Pollution Survey, an assembly of existing information on industrial wastes, scattered through the literature, and its organization in the form of preliminary industrial waste guides, was begun early in 1939, at the start of the survey. To Mr. LeBosquet should go the credit for conception of the uniform guide type of presentation.

The initial usefulness of the guides was to assist the field engineers in inspecting and reporting the various industrial discharges in the Basin, to furnish the office with the necessary information for estimating waste loadings and in the determination of remedial measures and their costs.

The preliminary guides also furnished the means for approaching various authorities active in the industrial waste field over the country, inviting their criticism and supplemental information.

Later on the guides were revised to incorporate the additional data offered by such sources, and the additional information developed during the survey itself, and the final drafts were printed as Supplement D to the Ohio River Pollution Control Report. (H.R. 266, 78-1)

The eleven guides so prepared represent the more common industries in the Basin, namely:

Brewery
Cannery
Coal Washery
By-Product Coke
Cotton
Distillery
Meat
Milk
Oil Refinery
Paper
Tannery

Other industries represented in the Basin include:

Chemical
Steel
Lead and Zinc Mining
Ore Reduction
Railroad Yards
Clay Products
Rendering
Glass Works
Wood Preserving
Rubber and rubber Goods
Paint
Natural Gas Works
Lumber Mills
Rag Cleaning
Vinegar Works
Limestone
Sand and Gravel
Candy
Plating
Tobacco
Soap
Fertilizer
Salad Oils
Phosphate Mining
Tannin Extracts
Rayon
Wool
Hosiery Mills
Glue Factories
Dye Works

In all, the field engineers turned in reports on some 1800 industrial waste producing plants during the survey.

The distribution of 1604 industrial plants not connected to existing municipal treatment plants, not including Cincinnati (information on total discharge from Cincinnati industries was obtained from available results of extensive sewer sampling and gagings) was as follows:

Milk	253	Paper	59
Canning	218	Oil Refining	47
Steel	174	Brewery	38
Meat	173	Tanning	32
Textile	122	By-Product Coke	23
Distillery	67	Miscellaneous	333
Chemical	65		1604

Figure 1, following page 1038, Supplement D, Industrial Waste Guides, indicates the geographical location of classified industrial plants in the Ohio River Basin, for which industrial waste corrections were suggested.

Table 2 of the same supplement shows the status of industrial wastes as found, their discharges to private or public sewers, and the number of plants having at least some corrective measures, most of which were minor.

The Industrial Waste Guides were prepared on a uniform pattern as follows:

1. Abstract
2. Description of Process
3. Raw materials , Product, Employees
4. Sources and Quantity of Wastes
5. Character of Wastes
6. Pollution Effects
7. Remedial measures including those within the plant
8. Bibliography
9. Inspection report
10. Note taking form
11. Flow diagram

I would like to run through one of the guides as a sample. As you have been furnished copies of the guides, I see no reason to review them all in view of the limited time. Instead, I would like to present later on some material on costs of remedial measures for some wastes, as worked out for application to plants in the Ohio Basin. An idea of the costs of remedial measures is an important part of a report on pollution control, and if you have ever had occasion to hunt for industrial waste treatment costs in this connection, you know that such figures are scarce.

As for sources of material drawn upon for the Guides, the search included the literature, correspondence with engineers and chemists in public and private agencies, contacts with equipment manufacturers, trade associations, industries, the Public Health Service files, the survey reports, and the results of sampling and gaging studies made in cooperation with the State of West Virginia, Tennessee Valley Authority, and the City of Louisville, on 29 plants.

You will find that considerable information in the industrial waste field is not published. For one reason, industry is understandably reluctant to broadcast any statistics on production. In others, the data obtained serve their specific purpose, and time is seldom available to work up the material for publication. For instance, Dr. Mohlman of the Chicago Sanitary District conducted 2-week, 24-hour composite sampling and gagings on something like 150 to 200 plants, on a wide variety of industries in the Sanitary District, and was hoping, back in 1939, that he could put on a man to work up the data for release. So far as I know the meat packing and brewery data were completed and published, but I have not seen the other valuable material in print. We were given the opportunity to use some of this unpublished material in connection with our work.

You will find in the bibliographies of these Guides fruitful source of material. They do not include a voluminous list of sources which were run down to turn out to be unproductive of useful data. You are probably familiar with Eldridge's work and publications, from which we drew heavily, and vice versa. Another later source of assembled material was a Digest of Industrial Waste Treatment, by C. I. Siebert, Pennsylvania Department of Health in 1940. The Annual Industrial Waste Conferences at Purdue, which started in 1944, promise to be a fruitful source of current developments in the industrial waste field.

(The lecturer went over a sample Guide with the Group)

This Station is of course interested in resuming the work on the Industrial Waste Guides to keep them current, and to expand the coverage to other industries. This will probably be one of the first assignments in connection with the possible setting up of an industrial waste section in the future.

Turning now to the cost of remedial measures, the table following shows the itemized cost estimate for the water pollution abatement program in the Ohio Basin. In considering Ohio River Report cost data, it should be kept in mind that these were based on average cost experience from 1928 to 1940, and that present day costs are much higher. It will be noted in this connection that the estimated cost of the additional capacity required in municipal treatment plants to treat industrial wastes was $13,100,000, and the additional costs for independent waste treatment on other corrective measures was estimated at $13,600,000. These figures do not include such important items as:

(a) Important Research Projects.

(b) Corrective measures resulting from (a) where specific recommendation could not be made.

(c) The expensive but necessary separation of organic and other polluting wastesfrom cooling waters.

Cost Summary - Ohio Basin

Treatment plants - for municipal sewage only	$61,900,000
Additional capacity for industrial wastes	13,100,000
Total cost of municipal plants	75,000,000
Interceptor sewers, to municipal plants	85,330,000
Total municipal costs	160,330,000
Cost of treating or otherwise correcting industrial wastes	13,600,000
Cost of mine sealing program	16,000,000
	$189,930,000

In order to estimate the cost of industrial waste correction for the large number of plants involved in the report on Ohio River Pollution Control, a review of sources of cost information, very fragmentary at best in the industrial waste field, was made. Following this a number of cost curves for various corrections were prepared for office use. A discussion of procedures and corrections, together with tables of costs developed for several of the more numerous types of wastes in the Ohio Basin, was prepared for Committee information, but was not published with the Report. As industrial waste correction cost estimate data are seldom available for a range of capacities, this material may be of particular interest, and is presented for that reason.

In considering the material which was prepared in 1941, the reader should bear in mind, first, that more recent developments in waste control processes may render obsolete some of the control methods outlined therein, and second, the present day costs are much higher than those used in preparing this material.

NOT FOR PUBLICATION

Presented in connection with a lecture at the U.S.P.H.S Sanitary Engineering Training Course in Stream Sanitation Practices, Cincinnati, Ohio, March 29, 1948.

METHODS FOR ESTIMATING COSTS
OF
INDUSTRIAL WASTE CORRECTIVE MEASURES

Prepared in connection with the estimating of costs of corrective measures, Ohio River Pollution Control Report.

S. R. Weibel

U. S. Public Health Service
Office of Stream Sanitation
Cincinnati, Ohio

October 1941

Revised November 3, 1941

FOREWORD

Considering the date at which this material was prepared, the reader should bear in mind, first, that more recent developments in waste control processes may render obsolete some of the control methods outlined herein, and second, that present day costs are much higher than those used in preparing this material.

INDUSTRIAL WASTE CORRECTIVE MEASURES

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SECTION TWO

Methods for Estimating Costs
of
Industrial Waste Corrective Measures

October, 1941, Revised November 3, 1941

General

Cost estimates on industrial waste corrective measures are based on recent cost experience wherever possible. In other cases, cost figures are engineering estimates based on inspections or available descriptions of industrial waste treatment plants. The experience and advice of industrial representatives and equipment manufacturers have assisted in arriving at many of the estimates.

In actual industrial waste treatment practice, each industrial plant receives a detailed study. This condition is approached in the present survey as the inspection reports cover most important features in sufficient detail that each plant can be given individual consideration. However, no effort has been devoted to estimating the cost of separating inoffensive cooling and other water from concentrated wastes, a customary step preliminary to installation of the industrial waste treatment plant.

Corrective measures applied to industrial waste pollution may include one or more of the following procedures:

> (1) Changes within the plant to reduce or eliminate the pollutional character of the waste. This can be done by changes in process, development of by-products, and by careful operation.
>
> (2) Treatment with domestic sewage in a municipal plant subsequent to possible pretreatment at the industrial plant and the exclusion of wastes detrimental to sewerage structures and treatment processes.
>
> (3) Treatment of the wastes at the mill prior to discharge to the stream. In some cases the cost of treatment may be substantially reduced by an effective application of procedure (1) above.

Plant Changes

Many opportunities for an economic solution of the pollution problem lie in the reduction of volume and strength of waste by revision of plant waste disposal practices, changes in processes, development of by-products, and reuse of wastes or recovered materials in the plant. At least a partial relief from the expense of correcting pollutional waste discharges should be possible.

While it is not possible to analyze all of the possibilities with respect to changes within the plant, some of the more common by-product recoveries and process revisions to reduce the volume of wastes have been taken into consideration in determining details and cost of corrective measures. These possibilities are covered in later paragraphs under individual industries. Undoubtedly any concerted movement toward an improvement of stream conditions will encourage the development of many additional methods for correction of wastes within the plant.

Treatment in Municipal Plants

Ordinarily, many industrial wastes can be treated more economically, and in some cases more effectively, at a municipal plant because of the economies and control exercised in larger scale operation. Barring complications in industrial plant-city relations, this solution is the most practical from an engineering and cost standpoint. Wherever industrial wastes are considered as acceptable and accessible to municipal treatment facilities they have been included as part of the cost of municipal treatment. In considering the acceptability of wastes for municipal treatment, due consideration is given to the size of the municipal plant, the type of treatment and the character and volume of industrial waste. Certain wastes, either chemical, organic or inorganic, are excluded from municipal sewers because of possible damage to sewerage structures - such as acids, impairment of treatment processes - poisons such as cyanides, or heavy metals; clogging of sewers or overloading of sludge handling equipment, or caking of sludge, - such as limes, hair, fibre; or concentrated organic wastes which may overload the plant. In many cases discharge to municipal treatment is made possible through the partial or pretreatment of wastes at the mill to reduce these objectionable characteristics. Such costs are carried as an industrial cost, as are the required industrial connections to municipal sewerage systems.

Industrial Waste Treatment Plants

Industrial waste treatment in a number of industries has advanced sufficiently that effective methods are well illustrated by plants now in successful operation.

In general, the types of industrial waste treatment upon which the industrial cost estimates depend, are based on processes illustrated by successful installations on a commercial scale. This applies to many but by no means all of the wastes requiring industrial treatment consideration in the Ohio Basin. In some cases the type of treatment is not considered a wholly satisfactory solution but is applied as a partial correction pending the solution of technical and financial problems connected with more efficient treatment. Finally, there are some types of waste for which economical treatment methods must be developed. In such cases either a type of treatment based on experimental data or the general character of the waste has been estimated for cost purposes, or the broad policy that all discharges containing objectionable settleable solids be given plain sedimentation as a minimum has been applied.

Installation Costs

Contrary to the situation in regard to municipal sewage treatment, cost data on industrial waste treatment is scarce and fragmentary.

Data on performance and costs for various types of full scale industrial waste treatment processes were derived from industrial reports of the present survey, from a survey of published information and from solicitation of State health departments, consulting engineers, engineers connected with industry, and companies manufacturing or distributing specialized equipment such as evaporators and by-product coke plant equipment.

In the cases where data on actual costs were available (in about half the cases, one for any one type of treatment), engineering cost estimates for the particular type of treatment were made for several capacities and the resulting curve relocated to compare with the actual cost available. In other cases, cost ranges were established from curves based on municipal primary or secondary treatment plant costs adjusted to the available actual industrial cost.

Where no actual cost data were available for representative treatment practice the costs presented are engineering estimates. Such cost estimates are based on the best available data obtained from a review of published contract unit prices, estimates obtained from manufacturers and other published or private sources of cost experience.

The construction cost figures obtained as above were adjusted to total cost by assuming, as has been done for municipal treatment plants, that land, engineering and miscellaneous expenses amount to 15% of the installed construction cost. Actually, land may be available at little or no added cost at many plants while at others this item may be a major factor in selection and cost of treatment. Similarly, engineering services may range from the employment of consultants, to the use of engineering services within the company or industry. However, sufficient data on individual plant circumstances are not at hand to warrant a study of possible variations in this 15% figure and it is considered reasonable for the general case. Contracting profits are also included in the basic construction costs.

Annual Charges

Interest and Depreciation - Fixed charges, including interest and depreciation or amortization on total first cost, is based at most plants on 5% interest and a life expectancy of 10 years. Some exceptions to this are covered in the detailed discussion following.

Operation and Maintenance - Data on industrial treatment plant operating and maintenance costs are very meager. Actual cost data has been used where such information is available. Operating and maintenance costs, in other cases, have been based on municipal treatment costs for similar sized installations with adjustment for chemical costs. In still other cases, independent estimates have been made.

Operating and maintenance costs are presented on a daily basis and applied in general to the actual number of working days annually indicated in the inspection reports. Where chemical precipitation is applied, it may be possible to reduce chemical costs during high flow periods. No attempt has been made to anticipate such savings in the annual costs estimated.

Corrective Measures and Costs by Industries

Detailed studies have been made of all available information on corrective measures and their cost for the common and most numerous classified industrial wastes in the Ohio River Basin. The costs of such measures are summarized graphically in Figures I-1 to I-5.

Discussions of measures and costs for breweries and tanneries which are numerous enough to be included in the above grouping are omitted. In the case of the breweries in the basins, locations in the larger municipalities make it possible to handle wastes in a municipal treatment plant. Practically all of the tanneries of large size now have varying degrees of treatment and the smaller ones are within reach of adequate suggested municipal treatment facilities. Such individual instances of industrial treatment or pretreatment as are required are handled as special cases. Otherwise, the measures assigned consist largely of supplementary equipment and plant improvements.

There are other miscellaneous industrial wastes in the basin, some of which, though they may be generally classified by the nature of their business, cannot be so classified for waste correction because of the wide variation in the character of wastes produced and their individual significance as governed by stream circumstances. These are handled as individual treatment problems with an application of corrective measures, including variously screening, sedimentation, flotation, recovery, separate disposal and chemical or biological treatment to part or all of the waste of the individual plant. The measures applied are based largely on available reported corrective practices, proposals or estimates by consultants or industry for the individual plant, or in some cases on measures suggested as a result of reported laboratory or plant investigations. No attempt is made to present a common denominator of cost for this miscellany of individual corrections.

Cannery

Industrial treatment methods for cannery wastes are well represented by numerous commercial installations. The processes in common use include screening, plain settling, chemical precipitation, ponding and broad irrigation, all of which are represented by installations in the Ohio Basin. Secondary treatment on trickling filters is commonly mentioned in the literature but is not widely represented by practice because of the high cost and the necessity of conditioning before seasonal use. Judging from the extensive use of ponding noted in the survey the problem of space appears to present no difficulties for the small rural cannery and the economy of such treatment presents an attractive opportunity for effective control for the short season cannery. One of the chief drawbacks to ponding is the creation of local odor nuisance. Considerable promise is reported in early season results of the application of sodium nitrate to cannery ponds being tried this season for the control of odor nuisance. While the cost of such control is appreciable, it offers the short season cannery a generally less expensive method of correction than a filter plant in the cases where a high degree of treatment is warranted.

Fill and draw chemical precipitation is also a common type of treatment and is particularly well represented in Wisconsin with over 50 plants. Reasonable in cost and effective in its applicability to most cannery wastes, it appears to offer a reasonable solution for the average case and has been adopted for cost purposes. Where a higher degree of treatment appears necessary, pond capacity equivalent to one-half the seasonal flow has been added to provide additional reduction in the load as discharged, by oxidation, seepage, and controlled outflow. An installation including chemical precipitation and 15 days ponding followed by a series of ditches, operates during a six weeks season of corn and tomato packing at Mason, Ohio. An overall reduction in B.O.D. loading of 90 per cent is shown by a single analysis taken during the tomato pack.

Construction costs for fill and draw chemical precipitation based on mechanical screening and a 2-hour cycle of mix, coagulate, settle and discharge in a two or more tank plant, with sludge drying beds, were estimated for several installation capacities at an assumed maximum discharge rate of 50 gallons per case per 12-hour day. The resulting curve was adjusted to the upper figure of $3,000 to $4,000 supplied by the Wisconsin State Health Department as the cost for a two-line plant. Ponds following chemical precipitation for further control were estimated at unit prices for 2-foot water depth, full.

Operating and maintenance costs for chemical precipitation are based on data presented in Bulletin No. 28-L of the National Canner Association (Dec. 1939). Pond operating and maintenance costs are considered negligible. In cases where odor nuisance is likely to be of consequence, odor control by sodium nitrate or other means, or trickling filter treatment, should be considered.

The estimated costs, including 15 per cent of the construction cost for land and engineering, are as follows:

Production Lines	Production Cases per day	Average Waste Flow Gallons per 12-Hour Day	Total Installed Cost Chem. Precip.	Total Installed Cost Ponding 15 Days	Total Installed Cost Ponding 30 Days	Daily Operation and Maint.
1	500	12,500	$2,900	$1,000	$1,300	$8.00
1	1,000	25,000	3,500	1,300	1,800	9.50
2	2,000	50,000	4,600	1,800	2,400	13.00
3	3,000	75,000	5,800	2,100	2,900	16.50
4	4,000	100,000	6,700	2,400	3,300	20.50
5	5,000	125,000	7,500	2,700	3,700	24.00

The above costs may be applied to the individual plant on a line, case, or flow basis, preferably flow, depending on data available, and adjustments made for individual plant characteristics.

An overall treatment removal of about 50 per cent in biochemical oxygen demand loading may be anticipated with properly applied fill and draw chemical precipitation, and upwards of 75 per cent when followed by ponding to regulate discharge to one-half or less of the seasonal inflow rate. In assuming pond capacity no allowance has been made for oxidation and seepage.

Milk Plants

It is not proposed under any method of treatment, industrial or municipal, to provide for intentional discharge of whey, buttermilk, skimmed milk or whole milk. The development of by-product uses and separate disposal to farmers or land has now become a common practice in the Ohio River Basin as elsewhere.

Processes of treatment in common use at milk handling and processing plants include septic tanks, trickling filters, activated sludge, Guggenheim process and broad irrigation. All of these are represented in the Ohio River Basin. There is also a cream, casein, whey powder plant in the basin which disposes of casein and whey plant wastes to leaching gravel beds and treats creamery wastes by chemical precipitation using lime, marl and copperas.

The bulk of the milk plants surveyed, particularly the large plants, are so situated so that they will be accessible to municipal treatment. The plants remaining for industrial treatment considerations are substantially small cheese factories and small to medium miscellaneous milk plants.

The trickling filters appear to be the most suitable and common method of effective correction for installations in this range. Installation costs are generally higher than for the other refined processes but the dependability and relatively simple and economical operation of the trickling filter makes it generally preferable.

Several ranges of trickling filter installation costs were available from the literature, the Wisconsin State Health Department and the Carnation Company (which acts as engineer and contractor for its many trickling filter installations throughout the country). These, together with estimated costs for several capacities were used to determine a curve of construction costs to which the 15 per cent land and engineering adjustmemt was added. Operating costs are estimated with the guidance of some actual costs in the upper ranges.

The resulting costs are:

Milk Intake (1) Pounds per Day (Flush Season)	Estimated Average Waste (2) Flow to Treatment Gallons Per Day	Trickling Filter Plant Costs	
		Total Installed Cost	Daily Operation and Maintenance
20,000	3,200	$4,000	$1.50
50,000	8,000	5,500	1.92
100,000	16,000	8,000	2.60
200,000	32,000	11,500	3.84
300,000	48,000	14,500	5.20

(1) Generally applicable for all types of plants except cheese factories. In applying cost table to cheese factories the installation cost corresponding to the actual flush season cheese factory intake should be approximately doubled (to provide for the additional filter capacity required on a B.O.D. loading basis). No such adjustment for operating cost need be made since the average cheese plant waste flow approximates those given in the table, so that pumping, etc. would therefore be the same on an intake basis.

(2) Does not include inoffensive cooling waters, nor contemplate treatment of any products or by-products except those incidental to washing operations.

Since mahy of the milk plants, even though small in size, discharge to ditches and small watercourses, secondary treatment equivalent to the above is required to abate local nuisances. In some cases the cost applied represents the addition of filters to existing septic tanks. There are a few plants, however, discharging to streams of sufficient size that correction by primary treatment appears sufficient. In such cases the cost of sedimentation is assigned.

Paper and Paperboard Mills

A review of sources of information on the treatment and disposal of paper and board mill wastes indicates that the bulk of reported actual practices revolve around the saving of fibre from machine wastes. At paper mills working with clean raw materials, or at most board mills, recovery and recirculation, or recirculation alone may constitute an effective means of reducing the obnoxious effects of the raw wastes. The recovery of fibre and partial recirculation of machine wastes back to the process is probably as common a practice in the Ohio Basin as elsewhere for similar mills. However, many of the mills in the basin include in their process the preparation of rags, rope and old paper. Mills processing dirty rags as part of their paper making operations may have wastes from rotary boilers, washers and beaters constituting in the neighborhood of 70 percent of the total population equivalent B.O.D. loading as compared with the 30 per cent machine waste loading usually discussed in treatment references.

A review of actual practices discloses methods of correction as follows:

Save-alls, Recirculation - Screens, vacuum filters, flotation and sedimentation devices on machine wastes at paper and board mills are common. Recirculation of machine wastes with or without save-all clarification is also common. Reported practices at three Pennsylvania board mills effected reductions of from 39 per cent to 85 per cent of the population equivalent (biochemical oxygen demand) discharge loading by save-alls plus recirculation in a partially closed system and 90 per cent and over at three other board mills having closed systems and sedimentation of weekly or other intermittent cleanup wastes. Reductions at three Pennsylvania paper mills were reported as 64 per cent, at a mill making fine paper and using a save-all and partially closed system; 50 per cent by the same method at a tissue mill; and 79 per cent at a towel mill.

Many of these practices are represented at Ohio Basin mills. In some cases where the stream is small and the mill large, or where mills concentrate in a short stream section, the residual waste loading from these practices becomes of consequence. In general, it appears that these practices can be applied with high overall efficiencies to board mills where suspended matter can ordinarily be returned to the product, and to paper mills working on clean raw material such as wood pulp, paper shavings and clean rag stock or pulp, in contrast to those cooking dirty rags or rope or de-inking and pulping waste paper, or those having color change complications. In the case of the latter, recirculation and recovery as commonly applied effects a machine waste correction only. The correction of wastes from rotaries, washers and beaters becomes a treatment problem with little prospect of economic recovery at this time.

Chemical Precipitation - Chemical precipitation applied to a Pennsylvania board mill gave a 70 per cent improvement in population equivalent discharge loading.

A Michigan mill making tarboard automobile strips on a wet machine and boxboard, wrappers, asbestos board, etc., on a dry machine treats these wastes separately and returns the sludge to the respective process. Biochemical oxygen demand reductions of 63.5 per cent and 63.8 per cent for the wet and dry machine wastes respectively, were obtained.

An Indiana mill manufacturing grease proof, glassine, and manifold paper from sulphite pulp treats all wastes with alum in the first of three ponds. Sludge is dried on beds. Pond effluent is recirculated to the process. The replacement cost of the system including save-alls is estimated at $50,000. Production is 18 tons per day.

Ponding - An Ohio board mill producing paper board from wood pulp, paper shavings and newspapers (de-inked at mill) discharges beater washes to a seepage pond. Machine wastes are recirculated.

Another Ohio board mill using wood pulp, paper shavings and waste paper, provides pond detention for 10 per cent of the machine waste and part of the sludge from multiple sedimentation of 90 per cent of its machine wastes. The bulk of the sludge is returned to the process. About 80 per cent of the waste water is recirculated.

A large Pennsylvania pulp and paper mill has provided a reservoir having a capacity 60 times the daily discharge of its de-inking wastes and wash waters from the used paper department. The pond effluent discharges 1.4 miles down a steep channel to the river. The raw wastes average 60 p.p.m. B.O.D. and the wastes entering the river about 14 p.p.m. or an overall reduction of 77 per cent.

Filtration - No mills at present using filtration have been noted. Two Massachusetts mills which have discontinued filtration since the extension of the Boston Metropolitan District Sewer, had treatment facilities as follows:

The one manufactured manila and other paper from rope, bagging, and wood pulp. Machine wastes were recirculated to the washers. Cinder filters received all the settled washer waste and as much of the settled beater waste as they would take. Rotary liquor was discharged either on the drying sludge or on the cinder filters with a lowering of their efficiency. Operation subsequent to connection to the Metropolitan Sewer consisted of discharge of oily rope beater wastes

(3) beaters) to the Sewer and discharge of the settled waste from 19 other beaters to the river. Sludge was discharged to the sewer. Save-all material was burned.

The other mill produced heavy paper boxes, shipping cases, cartridge papers, etc., from cotton and hemp stock. Machine wastes were settled with alum by fill and draw operation with a return of sludge to the process and discharge of the effluent to the stream. Boiler liquors were discharged to waste land. Washer waste was plain settled and filtered on sand. Sludge was dried in beds. Practice subsequent to the extension of the Metropolitan Sewer was the discharge of all but machine wastes to the sewer. Treatment of the machine waste as above was apparently continued.

Miscellaneous Treatment Information - Comments on rag and de-ink wastes by C. L. Siebert, Pennsylvania Department of Health, are as follows:

> "A few mills in Pennsylvania produce pulp by digestion of rags or old rope with a caustic solution. The wastes from such a process are extremely high in pollution load. Two small mills are entirely disposing of rag digester wastes by discharge to leaching pits, but, because of the jelly-like deposit produced, it is doubtful if such method will prove permanently satisfactory unless occasional complete cleaning or construction of new leaching basins is a practical solution. One mill attempted to evaporate digester wastes for recovery of caustic but found that forming in the multiple effect evaporator could not be successfully controlled. A mill which digests old rope, after indifferent results with acid coagulation, has met with rather marked success by calcium chloride coagulation

of the wastes in batch of fill-and-draw treatment, with absorption of the clarified or supernatant liquor in loose gravel soil and drying the settled sludge in a sludge bed in natural formation.

"A number of paper mills de-ink magazines and similar used paper to produce a pulp which is mixed with new pulp in the manufacture of magazine and some book paper. The de-inking process involves the use of strong chemicals such as caustic soda and the waste waters produced are very high in pollution load. No conventional method for treatment of these wastes appears to have been developed, but chemical coagulation and sedimentation have been shown to be effective although expensive. At Johnsonburg the de-inking wastes and wash waters from the used paper department are impounded in a reservoir having a capacity 60 times the daily discharge and the effluent flows thence 1.4 miles down a steep channel to the river. The raw wastes average about 60 p.p.m. B.O.D. and 250 p.p.m alkalinity (M.O.) with pH values between 7.0 and 8.0 The wastes entering the river average about 14 p.p.m. B.O.D. and 6 p.p.m. D.O."

Adoption of Corrective Measures for Cost Purposes - In considering the practical aspects of assignment of specific corrective measures, it becomes evident that the general lack of application of available corrective measures in practice to wastes other than those from which an economic return may be expected, and consequently the scarcity of loading and performance data, considerably handicaps an effort to assign and anticipate the degree of performance of available measures for many types of mill, particularly those processing rags and old paper. It is also evident that in places where a high degree of correction appears warranted, the cost of correction may range from one extreme to the other dependent, not only on the type of mill process, but on the choice of available corrective measures.

While recognizing the possibilities of a high degree of correction either by large ponding capacity and controlled outflow, or by intermittent sand or cinder filtration for individual cases, it has not been considered feasible to specify these measures and their divergent costs for the following reasons.

Sufficiently detailed data on mill locations, topography, space and other local contingencies are not available within the scope of the survey to propose large ponding facilities at a speculative cost for the specific solution; in the cases where local conditions permit it, ponding with a program of effluent control synchronized to the characteristics of the particular stream should be an economical solution. The problems of space and local environment inherent in pond considerations apply equally to intermittent filters as a specific proposal. In addition, the cost of such treatment is such that an extensive examination of economic possibilities in process changes at the particular mill and detailed analytical study of the receiving water, mill wastes and pilot performance of other less expensive facilities, including those now applied to varying degrees, as applied to the particular mill, would be warranted. Since such a study for the individual case is not available the proposal of expensive intermittent filters as a specific solution also appears unsound.

As an alternative to the specific proposal of ponds or filters and their costs for such cases requiring a high degree of treatment it is proposed that the cost of partial correction included in the report be considered an initial step pending investigation of specifically applicable refined measures. The foregoing applies to the few cases where the character and size of the mills and stream circumstances are such as to warrant a high degree of control. In most other cases the corrective measures indicated in the following paragraphs should provide an effective solution.

The corrective measures adopted for cost purposes and based so far as possible on the practices aforementioned, supplemented by information presented by engineers interested in this field, are as follows:

The revision of machine piping and appurtenances to recirculate all or as much as practical of the machine wastes back to the process. Apparently board mills so corrected may be operated with reduced waste flows ranging from 14,000 gallons per ton to substantially zero in a closed system with weekly or other intermittent cleanup discharges, while paper mills can, by partial closure, greatly reduce the volume of waste discharged. Data collected in Wisconsin paper mill studies "....bears out the contention that most any paper mill can operate with a fibre loss to the sewer not exceeding 1 per cent (of production) and a waste flow of 20,000 gallons or less per ton of finished produdt". C. L. Siebert, Pennsylvania Department of Health, is convinced that 10,000 gallons per ton and 1/2 per cent fibre loss is possible without hardship. It is proposed for estimate purposes that the average board mill reduce its residual discharges by recirculation revisions to at least 10,000 gallons per ton and the paper mill to 20,000 gallons per ton. The cost of machine revisions for recirculation obtained from C. M. Baker, Consultant in the paper field, is $4,000 per machine. The figure $5,000 per machine is applied to plants in the Ohio Basin as the complete cost of this correction. In cases where the individual plant report indicates recirculation and reduction of flow in the range of those proposed, the cost is not applied.

As indicated by performances at three Pennsylvania board mills the population equivalent (B.O.D. basis) loading residual to a partially closed system averages about 25 P.E. per ton of board, or a reduction of about 73 per cent of the initial 93 P.E. average for these mills. Since the unit loadings assigned board mills of the Survey were about 100 P.E. (B.O.D.) per ton the residual of 25 represents a 75 per cent reduction. In many cases this offers an acceptable solution for the board mill from a B.O.D. standpoint. With respect to fibre losses (as volatile suspended solids) the percentage reductions surprisingly are lower, particularly where no save-alls are used. In some cases local conditions as regards sludge bank formation may necessitate further correction in this respect. Facilities for chemical precipitation during critical low flow months and plain sedimentation the remainder of the year appears to be a feasible method of reducing the pollution effects of continuous residual discharges, or intermittent discharges due to cleanup or stock dumps on color changes.

The same considerations substantially apply to paper mills working on clean raw materials. Judging from the results of recirculation at three save-all equipped Pennsylvania paper mills manufacturing towels, fine paper and tissue respectively, and having partial to almost complete closure, considerable reduction in B.O.D. loading (50 to 79%) is possible, Percentage reductions in fibre losses were more comparable on the average to those of B.O.D. loading. Consequently, recirculation may constitute an acceptable solution in some cases. However, since many of the Ohio Basin mills pulp rags and old paper as part of their process, the residual washer waste loadings are likely to be high because of pulping wastes, regardless of the disposition of rotary liquor and stock drainings. For such mills facilities for plain and chemical sedimentation appears to offer the best general solution. While B.O.D. removals in the sedimentation units as applied to the partially corrected wastes are not expected to be large, the facilities offer relief from washer and beater suspended matter and provide safeguard against direct discharge during system cleanups and stock dumps on color changes. Chemical precipitation is one of the two proposals for the solution of de-ink waste problem noted thus far, the other alternative being long period impoundment.

Where mill processes include rag cooking there appears to be no industrial treatment process, except ponding thus far noted in which boiler liquor and stock drainings can be accepted along with other wastes without harm to the efficiency of the process. Separate disposal to land, to drying sludge or intermittent filters, to leaching pits, and to fill and draw chemical coagulation with calcium chloride followed by soil absorption, sum up the industrial disposal possibilities with present knowledge. For the purposes of this report it is assumed that boiler and drainer wastes may be discharged to drying sludge at no additional disposal cost. In some instances these relatively small volumes of waste could probably be taken into a municipal system as is now done at Dayton, Ohio.

Summary - A tabular summary of the corrective measures for paper and paperboard mills adopted for the purpose of estimating costs of remedial measures and the basic costs of these measures are as follows:

Corrective Measures

A. Board Mills

(1) Partial Correction - Recirculation.

(2) More Complete Correction - Step (1) followed by chemical precipitation on the basis of assumed 10,000 gallon per day residual waste per ton of product.

B. Paper Mills

(1) Partial Correction - Recirculation - confined as a satisfactory partial correction to mills not cooking rags nor cooking or de-inking old paper.

(2) More Complete Correction - Step (1) followed by chemical precipitation on the basis of assumed 20,000 gallons per day residual waste per ton of product. Suggested as a first step pending investigation of more efficient processes in some cases.

Estimated Costs

Recirculation improvements - $5,000 per machine installation cost. It is assumed that the saving in water and materials will at least defray operating costs. The total charge to pollution abatement is represented by fixed charges.

Chemical Precipitation

Mill Production Paper Tons/day	Board Tons/Day	Corrected Waste Flow M.G./24 hrs.	Total Installed Cost	Daily O. & M. *
25	50	0.5	$30,000	$19.
50	100	1.0	45,000	33.
100	200	2.0	70,000	60.
250	500	5.0	155,000	143.

* Including cost of chemicals

VI.

Distilleries

Seventy-one distilleries were covered by the survey. Practice reducing the pollutional loading discharged to streams at over 80 per cent of the plants were reported as follows: Disposal of excess slop to farmers; to cattle penned in the vicinity for the purpose (pen effluents variously ponded, pumped to land or allowed to reach the stream); evaporation of slop; ponding or broad irrigation. Screens and dryers were in use at most plants. No other commercial practices in reduction of loadings have been noted, in the Ohio River Basin or elsewhere. Wash waters and tailings are commonly discharged to the stream and constitute substantially the total pollutional discharge at some plants.

The remedial measures proposed for cost considerations are (1) evaporators at large distilleries and (2) ponding or disposal to land at small distilleries.

Evaporators - The evaporation of slop, tailings and washes at distilleries mashing 1,000 or more bushels of grain daily is proposed. It is claimed in some quarters that a profitable recovery results from evaporation, and in others, that the installation is not warranted on the basis of profit motive alone. In the case of the latter, it is claimed that because of the high cost of evaporator recovery the total net operating profit from investment in screening, drying and evaporator equipment would be the same as that which can be obtained from the dryhouse installation of screens and dryers only.

A reduction of over 95 per cent of the sewered population equivalent biochemical oxygen demand loading to the stream should result from evaporation if slop were originally discharged to the stream. For plants now disposing of slop other than to the stream the net reduction in population equivalent loading to the stream would be about 50 or 60 per cent, considering original wastes as wash and tailings, and the corrected wastes to be the distillate from slop, tailings and washes. However, in the latter case the resulting improvement over a period of time would certainly exceed 50 or 60 per cent since it was evident from the survey that cattle pen effluents could reach the streams and that slop discharge directly to the stream in times of lack of farmer demand or for other reasons, was likely, and not uncommonly evident.

The estimated cost of evaporator installations, including boilers, evaporators, additional dryhouse equipment, buildings and miscellaneous items based on manufacturers estimates are as follows:

Daily Operation Bushels Grain Mashed	Total Installed Cost
1,000	$ 55,000
2,000	80,000
3,000	100,000

Disposal to Land or Ponding - Evaporator installations for small distilleries are reported not practical and it has been assumed that other correction must be applied to distilleries mashing less than 1,000 bushels grain daily.

Disposal of slop by sale to farmers is a highly desirable and economical practice but has its limitations in fluctuating farmer demand and some objection to rye slop. In either case, at least an intermittent dumping of slop can be expected. In addition to this, the washings and tailings are not commonly disposed of in this way although one plant apparently puts all wastes in its sales tank.

Disposal of slop to penned cattle is another common practice which, while not too satisfactory from a local nuisance standpoint, does effect a considerable reduction in the slop B.O.D. loading judging from an incomplete sampling study in one case.

In each of these cases, an additional method of disposal as a safeguard to the stream appears desirable. While most of the small distilleries in the basin use one of these two partial disposal practices followed in some cases by pumping to land or ponds, there are several distilleries at which neither the above or other corrective measures are taken. Final disposal to land or ponds, possibly with outflow control, are the only methods illustrated by practice. This method, while widely represented in practice, has not been highly successful nor particularly desirable because of faulty design or poor maintenance resulting in washes and overflows to the stream and because of local odor and fly nuisances. Therefore, while this method is applied as a general correction for the purposes of the Report, it is done so with reservation and necessity because of lack of more desirable representative practice. No doubt in many cases local conditions will permit land or pond disposal to be effectively applied. In other instances where local contingencies preclude its effective application consideration might be given to the use of high rate recirculating single or multiple filtration, or the process of anaerobic decomposition. Pilot plant studies of both of these methods have been made with promising results.

The cost of land or pond disposal estimated for the general case includes facilities for pumping and delivering all wastes 1/4 mile to land or pond. In the absence of actual cost data, cost estimates of pumping and piping facilities were made to which were added the cost of prepared land. Allowable land loading was assumed as an average 10,000 gallons per acre per day and the cost of acquiring and preparing land and distribution system as $1,000 per acre based, because of lack of local cost data, on reported Berlin, Germany, sewage farm costs of $764.58 per acre for specially prepared land. The cost of ponds, considering percolation, pond oxidation and outflow control, to carry over most low flow periods, should not exceed the cost assigned to broad irrigation and the costs assigned are considered within the limits of the accuracy of the estimate to approximate either correction.

The costs presented herewith represent the correction for small distilleries discharging all wastes to the stream, including those not at present providing protection against sales slop discharges or cattle pen effluents. Since many of the small distilleries in the Basin report having facilities for such protection to varying degrees of satisfaction, the application of individual corrective measures and costs vary from improvements to existing practices to the complete correction indicated below.

The costs so estimated are as follows:

Distillery Operation Bushels Grain Mashed Daily	Cost of Land or Pond Disposal Total Installed Cost	Daily Operation and Maintenance
500	$3,600	$1.65
750	4,300	2.17
1,000	5,000	2.70

By-Product Coke Plants

Due to process methods, recovery practices and characteristics of the wastes, miscellaneous correctional measures are customarily applied to the separate wastes. For the sake of clarity in presenting methods and costs of correctional measures, the individual wastes produced by the usual by-product coke plant are listed below:

Waste	Amount G.P.D./Ton Coal	Characteristic
Ammonia Still	22	4000 p.p.m 5-day B.O.D. 2000 p.p.m. phenol
Final Cooler	315	220 p.p.m. 5-day B.O.D. 100 p.p.m. phenol
Benzol Carrying	15	650 p.p.m. 5-day B.O.D. 70 p.p.m. phenol.
Spent Acid	Small	
Spent Caustic	Small	
Pure Still Residue	0.6	
Quench to Atmos.	125	
Residual Quench	475	
Cooling Water	3200	

Based on limited available analytical data the population equivalent B.O.D. breakdown on the total wastes is as follows:

Waste	Pop. Equiv. (B.O.D. basis) per ton coal carbonized daily
Ammonia Still Waste	5
Final Cooler "	4
Benzol Carrying "	1
All other by difference	5
Total	15

A review of existing disposal practices at the plants surveyed in the Ohio Basin indicates that, with the exception of a few small plants and two large plants for which details could not be obtained, practically all of the plants report facilities or practices for at least partial correction of their waste problem. These measures are variously:

By-product recovery of Ammonium Sulphate, Tar, Benzol Toluol, Xylol, and coke breeze.

Phenol recovery at some plants, by either the Koppers process or the benzol extraction process.

Ammonia still wastes, variously: used for quenching, discharged to slag dump, distilled and the distillate containing phenol used for quenching - in this way corrosive salts are kept from the quench system.

Final cooler water is cooled and recirculated in a closed system with discharge to dump when high in solids. It is also used for quenching. The quantity of waste produced without recirculation, however, is apparently much larger than that volatilized in quenching.

Quench water practices vary from the use of the plant water supply to the use of quench substantially made up of waste, including variously, ammonia still waste, benzol plant waste, final cooler water, and agitator wastes. Some quench systems are entirely closed with recirculation of residual water and recovery of coke breeze.

Acid sludges and benzol still residues are burned, discharged to dump, or used for quenching.

At least one plant has a gravity separator on its benzol recovery unit.

Considerable attention has been paid to phenol and its reduction by recovery. In the Koppers vapor-circulation process as applied to ammonia still liquors, the phenol removals in the dephenolizer ranging from 80 to 96 per cent and overall removals of from 90 to 98 per cent are indicated in data available. The benzol extraction process applied to ammonia liquor ahead of the still gave an overall removal of 97 to 99 per cent at one plant. While the recovery of ammonium sulphate is a very common practice, the recovery of phenol for economic return alone does not appear to be borne out by common usage, although a dephenolizer at a large plant has been remarked as being installed for the purpose of phenol recovery and not waste disposal. In another instance an installation of the benzol extraction process was said to break even on operating costs through the sale of phenol but not on fixed charges. Since the ammonia liquor phenols probably constitute more than half of the phenols produced, the reduction is significant, but is apparently not a complete correction. The other large phenol bearing waste, final cooling water, appears to be too large in volume to be entirely dissipated in industrial coke quenching. Correction of this waste by partial or complete recirculation offers a reasonable correction and is in use at some plants.

The removal of B. O. D. from coke plant wastes has received less attention, with the result that little is known about the B.O.D. removals accompanying phenol recovery or other practices. It has been stated that B. O. D. removal by the benzol extraction process is considerably less than that of the phenol. However, practices such as recirculation of final cooler wastes in a closed system and the use of ammonia still wastes, and such other wastes as feasible for quenching in a closed system, should, by physical removal, effect a reduction of upwards of 60 per cent of the uncorrected total B.O.D. loading. This method appears feasible and is represented by actual practice where a non-domestic coke is produced.

In the case of domestic cokes, objections are raised to phenolic odors resulting from quenching with wastes. Experimental work with chlorine has resulted in the suggested treatment of either the ammonia still waste or the domestic coke with chlorine, with a conversion of phenol to trichloralphenol and a resulting dissipation of most of the objectionable phenol odors. The practicability of this method as regards substantial relief of all objectionable odor and the operating cost involved has not, as far as known, been demonstrated by actual practice. However the use of a phenol recovery unit on ammonia liquor wastes should offer a possibility in this respect.

For the purpose of estimating costs the composite correction is assumed to consist of the following:

(1) A closed quench system utilizing ammonia still wastes and as much other contaminated waste as practicable. The cost of this correction for a plant now quenching with fresh water and recovering breeze would be that of the construction of a small pump sump, connection to quench station sewer and reconstruction of the plant piping system to deliver wastes to the quench station. The latter item may be very expensive judging from one known case and may involve operating expenses where pumping is required. Individual plant details are not available to estimate such changes and this item is not considered in the expense of correction. Little increase in investment charges other than the collecting system should be involved in the correction because pumping facilities are usually required for the fresh water quenching system and the cost of a pump sump and sewer connection should be very small.

While operating costs, exclusive of those where pumping in the collecting system is required, should not increase appreciably, there would likely be an increase in maintenance costs. At one large plant quenching with wastes the maintenance expense is reported to average $5.48 per day per 1000 tons daily coal carbonizing capacity. The life of quench equipment has been estimated at 7 to 10 years using clean water and 2 to 4 years when using phenolic wastes for quenching. If the maintenance charges are assumed as inversely proportional to the useful life, the relative maintenance charges for quenching with wastes would be about 3 times those for fresh water quenching or on the basis of the above cost, an increase of $3.65 per day per 1000 tons daily coal carbonizing capacity. For the purposes of estimate it is assumed that, exclusive of the expense of a collecting system, the increase in investment is negligible and that the total additional charges are roughly $4.00 per day per 1000 tons daily coal carbonizing capacity.

(2) In the few cases where a domestic coke is produced the correction estimated for cost purposes includes a phenol recovery unit on ammonia liquor wastes. It is assumed that the operating and maintenance cost of the phenol recovery unit would be met by the return on recovered phenol but that fixed costs are chargeable to pollution abatement. The costs of such units estimated from cost approximations furnished by equipment manufacturers in this specialized field are as follows:

Tons Coal Carbonized Daily	Installation Cost Dephenolizer
500	$35,000
1,000	35,000
2,000	60,000
3,000	83,000
4,000	103,000
5,000	115,000

(3) A closed system with cooling tower on final cooler wastes. The total charges of such a closed system are assumed to be chargeable to pollution abatement. The estimated costs of such a system based also on limited manufacturers' cost approximations are as follows:

Tons Coal Carbonized Daily	Installation Cost Closed Final Cooler System	Approx. Daily O. & M.
500	17,000	$3.25
1,000	25,000	6.50
2,000	42,000	13.00
3,000	(58,000)*	19.50
4,000	(72,000)	26.00
5,000	(83,000)	32.50

* Extension of estimated costs.

(4) Separate disposal to land or burning for residues and waste components not feasible for use in the quenching system. No estimate of cost is included.

Some or all of the above measures are applied at present to many of the plants in the Basin and in most cases the costs applied are adjustments based on the above to allow for existing measures in the individual case.

Steel Industry

The disposal of acid pickle liquors is the problem considered in this discussion. Wastes from other plant processes are quite generally subjected to recovery or disposal practices which render them unobjectionable. In isolated cases considerable quantities of scale or flue dust may be discharged to streams; correction of such conditions will be priced on the basis of plain sedimentation. Wastes from the production of coke are discussed elsewhere in this report.

A number of processes for recovery of usable by-products from spent pickle liquor have been developed. All of the processes are expensive, and the demand for the products is definitely limited at present; consequently, it cannot be recommended that these recovery methods be universally adopted.

The simplest method of treating waste pickle liquors is by neutralization with lime or ground limestone followed by sedimentation, with no attempt made to recover by-products. A number of such plants are now in operation, and, while not entirely satisfactory, it is the most practical corrective measure available and is used as a basis for estimating the cost of treatment.

The plant, as roughly designed for cost estimation, consists of a storage tank for equalizing the flow of pickle liquor and rinse water; storage bin and crusher for limestone; solution feeders and mixing tanks with air injection and mechanical agitating equipment; and final discharge to one of two settling basins operated alternately with periodic shutdown for sludge drying and removal.

Cost estimates on plants of similar design were received at Cincinnati with industrial waste reports from two steel mills. These estimates were made by consulting engineering firms and were used in conjunction with equipment cost data to price installations for handling flows of from 20,000 g.p.d. to 300,000 g.p.d.

The estimated costs, including 15 per cent for contingencies and engineering, are as follows:

Ave. Waste Flow* Gals./24 hr. day	Total Installed Cost	Daily Operation** & Maintenance
20,000	$ 6,400	$ 3.50
100,000	13,700	4.30
200,000	21,000	7,00
300,000	29,000	9,50

* Includes pickle liquor and rinse water.
** Does not include the cost of limestone.

The quantity of limestone required has been computed from the amount of acid used since complete neutralization of the waste H_2SO_4 and $FeSO_4$, the equivalent of neutralizing all of the acid applied to the process, is contemplated in making the cost estimates. Assuming a limestone of 95 per cent purity and a quantity 10 per cent in excess of that theoretically required, the ratio of limestone to sulfuric acid is 1.2 to 1. The cost is estimated at $4.50 per ton including labor and power for grinding and the charges will greatly exceed those shown for operation and maintenance.

Most of the mills in the Ohio Basin will fall within the range 20,000 - 300,000 g.p.d. In very few instances the discharge of acid wastes may be in excess of 1 M.G.D. Because of the small number of such large plants, the cost curve has not been extended to include them. Each will be considered as a special case.

For flows of less than 20,000 g.p.d., the installation has been simplified to include only one tank which serves for both storage and mixing. Lime is assumed to be the neutralizing agent and will be fed to the tank by gravity. A simple propeller-type agitator is used to obtain thorough mixing. The neutralized solution may be discharged directly to the stream in most cases although extreme low flows might necessitate separate sludge disposal. Installation of equipment for this type plant can be made for about $65.00 per 1000 gallons of liquor treated daily. It is believed that lime will be cheaper than ground limestone for small plants and the quantity required is estimated from the amount of acid used, as for the larger plants. The ratio of lime to sulphuric acid is approximately 0.73 to 1. The system is designed for intermittent operation, requiring attention for only a short time each day, and power requirements are insignificant. The cost of lime ($15.00 per ton) may reasonably be assumed to represent the total operating cost.

Textile Mills

The survey indicated practically no industrial treatment of textile wastes in the Ohio Basin. Inspection reports on 139 mills in the Basin, including several manufacturing rayon, indicated the industrial treatment to be: neutralization, flocculation, or mixing, respectively, followed by sedimentation, at 3 mills manufacturing rayon; screening for fibre recovery at a woolen mill; and disposal by ground seepage at a small mill. Most of the mills are small and so located that they are accessible to, or are now connected to municipal sewerage systems in towns which can absorb the wastes in a municipal treatment plant without great difficulty.

Representative practices elsewhere indicate chemical precipitation with facilities for equalizing the wastes to be a common method of treatment, and one favored by various authorities. According to C. L. Siebert, Pennsylvania Department of Health, chemical precipitation as applied to textile wastes, in general, is considered to be capable of ordinarily removing 80 to 90 per cent of the oxygen consuming material from the stronger wastes (B.O.D. of 800 p.p.m. or over) and 60 per cent from the weaker wastes (B. O. D. of 200-300 p.p.m.). In some instances chemical precipitations have been supplemented by spray aerators, air jet mixing and flocculation, trickling filters and rapid sand filters.

Fill and draw chemical precipitation is generally applied to the smaller plants and would be applicable to about 80 per cent of the mills in the Ohio Basin not accessible to present or future municipal treatment facilities. The remaining 20 per cent, because of their size, would probably operate continuous flow plants.

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In the absence of representative actual textile waste treatment costs for chemical precipitation, engineering estimates have been made for cost purposes. Fill and draw chemical precipitation plants have been estimated on the basis of a 2-tank plant with a 5-hour cycle of mix, coagulate, settle and discharge. The continuous flow plant estimates include a 5-hour equalization tank followed by mechanical mixing, coagulation and 2 hours settling with continuous sludge removal. Sludge beds are included in both cases. The costs include 15 per cent of the installed cost for land, engineering, etc.; operating costs include chemicals.

Fill and Draw Chemical Precipitation

Flow Gallons per 10-hour day	Total Installed Cost	Daily Operation and Maintenance
10,000	$ 2,200	$ 5.20
25,000	3,100	6.75
50,000	4,600	9.30

Continuous Flow Chemical Precipitation

Flow Gallons per 10-hour day	Total Installed Cost	Daily Operation and Maintenance
100,000	$17,000	$13.00
250,000	30,000	24.00
500,000	43,000	43.00
1,000,000	75,000	80.00

Packinghouses, Slaughterhouses

With few exceptions the larger plants, packinghouses, in the Ohio Basin are so located that they can be connected to municipal systems and treatment. The bulk of the meat plants encountered were small slaughterhouses, some of which are not within reach of municipal systems and will require separate correction.

Filtration, multiple filtration, and activated sludge treatment are all represented by commercial installations for large meat plants. The cost of activated sludge treatment, in operation at the Kuhner Packing Company, Muncie, Indiana, is available. Removals at this plant for two 24-hour runs at 43 per cent design flow and 76 per cent design hog unit loading were about 98 per cent 5-day B.O.D. and suspended solids. The design capacities were 500,000 g.p.d. and 500 hog units, and the construction cost approximately $41,000. Operating and maintenance costs were about $13.00 per day (exclusive of fixed charges) over the first 6 months of operation. A range of costs based on the above was determined with the help of curves established for secondary municipal treatment plant costs in the Basin.

The resulting costs adjusted for land, engineering, etc. are as follows:

Design Flow Million Gallons per day	Total Installed Cost	Daily Operation and Maintenance
0.050	$13,800	$2.00
0.100	16,800	3.00
0.500	47,200	13.00
1.000	82,000	23.00

Cost of small slaughterhouse installations is based on fill and draw coagulation and sedimentation using chlorine and ferric chloride. According to E. F. Eldridge, this method of treatment has proven simplest and most effective for these small volumes of waste. A commercial installation was made by the Grand Rapids Packing Company. The cost was reported $500, exclusive of housing, for a design capacity of 5,000 g.p.d. Operating costs were not expected to exceed $1.50 per day at capacity. Removals of 88.4 per cent 5-day B.O.D. and 86.5 per cent suspended solids were obtained.

Total costs for several capacities were estimated for this process. The costs include pumping of waste and disposal of sludge to drying beds. The estimated installation costs appear considerably higher than the cost reported above, but since detail on the items included in the reported cost is lacking, no adjustment of the estimated installation costs have been made. The estimated operating costs were adjusted to conform to the expectancy noted above. The estimated costs are as follows:

Design Flow Gallons per Day	Total Installed Cost	Daily Operation and Maintenance
1,000	$350	$0.80
2,000	500	1.00
5,000	900	1.50
10,000	1,400	2.40

Corrective Measures and Costs - Miscellaneous

In some cases wastes discharging to the larger streams may be sufficiently corrected for the purposes of the particular stream by relatively simple measures such as screening, sedimentation, grease or oil separation, neutralization, and the separate disposal or treatment of parts of the waste having objectionable characteristics such as plating room wastes, etc. The same considerations hold where certain of the industrial wastes may be treated in a municipal plant after application of one of the above measures.

Measures such as flotation, neutralization and removal of salts or other miscellaneous objectionable characteristics are difficult of representation for general application to the variety of wastes and waste components encountered. These measures are applied as an independent estimate for each plant in the miscellaneous classification. In a few cases the cost of such correction is applied as an arbitrary judgment figure in the absence of sufficient detail on waste components. In most cases, however, the plant size, the probable volume of objectionable wastes, or the costs involved are relatively small and refinement in such estimates will have little influence on the cost of industrial waste treatment as a whole.

The estimated cost of the principal measures applied for partial or pretreatment are presented below.

Screening - Estimates of the cost of mechanical screening installations for cannery wastes are based on manufacturer's rotary screen ratings and costs, and on unit prices applied to pit, piping, and protective housing. About 70 to 80 per cent of the total cost represents the machine in place. In cases of basement installations this may represent substantially the entire cost. Operating costs are calculated on the basis of a cost given by L. V. Carpenter for a 100,000 gallon per day cannery as $10.00 per day for operation and disposal. The costs so estimated are as follows:

Average* Daily Flow Gallons per 12 hours	Total Installation Cost	Daily Operation and Maintenance
50,000	$1,500	$5
90,000	2,400	9
160,000	2,800	16

* Assumed as 1/2 maximum flow rating of machine

Plain Sedimentation - Installation costs estimated for plain sedimentation as a partial or pretreatment measure include concrete basins, sewage and sludge pumps, and appurtenances based on recent unit prices and manufacturers' equipment costs. Installations of various capacities and detention periods ranging from 2 to 24 hours were estimated, both with and without mechanical sludge removal apparatus. The total costs of such installations, including 15 per cent for land

and engineering for a range of flows at 2 hours detention are as follows:

Waste Flow Million Gallons per 24 Hours	Cost of Basin Without Mechanical Sludge Scrapers	Cost of Basin With Mechanical Sludge Scrapers
0.05	$1,400	-
0.10	2,200	-
0.25	3,800	$5,800
0.50	4,900	7,600
1.0	7,600	11,600
2.5	15,500	22,100
5.0	26,700	35,800

Operating costs have not been included above because of the wide variation in plant operating schedules which will materially affect both power and labor cost. Estimates of operating costs will be based on the quantity of waste to be handled and upon individual plant operation.

Ponding - Diked ponds applied variously for long period detention, or as an alternative in some cases to plain sedimentation in masonry basins, or as an adjunct to cannery or steel mill treatment processes have been estimated as square, formed by earth dikes 3 ft. wide at the top, side slopes 1 on 2, 2 ft. water depth with 1 ft. freeboard, priced at $1.50 per cu. yd. in place with a lump sum of $150. for inlet and outlet appurtenances to which 15 per cent for land and engineering has been added. Since local topography and land costs play such an important part in the cost of ponding facilities the costs are likely to vary widely. The total installation costs estimated as above, for a range of pond capacities are as follows:

Diked Pond Capacity at 2 ft. Water Depth Million Gallons	Total Installation Cost
0.5	$1,500
1.0	2,000
2.0	2,800
3.0	3,300
4.0	3,800

Connecting to Municipal Sewers - In many instances the cost of connecting to present or proposed sewer systems will be involved as a cost to the industry where municipal treatment for the industrial waste is suggested. An average distance of 500 feet and one manhole, is assumed for cost purposes.

The costs of such connections based on recent unit prices are as follows:

Rate of Flow 1000 Gallons per Hour	Total Cost of Connection
10 (and under)	$ 600
25	1,000
50	1,500

In the few known cases where pumping facilities are required, such cost is added into the connecting cost.

ORGANIZATION OF A STREAM POLLUTION SURVEY

(R. C. Palange)

1. Introduction

   a. Purposes of the Survey - to determine

      (1) Nature and extent of nuisance conditions
      (2) Suitability of waters for various uses

         (a) Domestic and industrial water supply
         (b) Navigation
         (c) Fish and Wild Life
         (d) Bathing
         (e) Recreation
         (f) Agriculture

      (3) Measures necessary for correction of the conditions
      (4) All other factors bearing on pollutional problems concerned.

2. Definition of the problems involved

   a. Contamination by domestic sewage - fluctuation of raw water quality imposes hazard to safety of a developed supply.

   b. Imparted chemical constituents by industrial wastes, both removable and non-removable by treatment

      (1) Excessive hardness - high soap consumption
      (2) Corrosion in hot water systems
      (3) Taste and odor contamination

         (a) Decreases value of supply for domestic use
         (b) Decreases use and revenue to municipality
         (c) Results in development of secondary public health hazards by persons seeking freedom from waters aesthetically offensive
         (d) Limitation of use in manufacture of food products, and, others

      (4) Results in extra cost to municipality for added treatment

   c. Special Problems

      (1) Acid Mine drainage
      (2) Soil conservation and navigation (turbidity and solids)
      (3) Pollution crossing boundary waters (state or international)
      (4) Ice coverage and seasonal fluctuations

3. Information Required

    a. Nature, location, and extent of pollution sources

        (1) Distribution of population and industries contributing pollution
        (2) Sewage and industrial waste treatment in the basin
        (3) Assemble all available information from different agencies

    b. Hydrometric factors

        (1) Stream and tributary discharges
        (2) Stream velocities - flow time, flow duration
        (3) Rainfall distribution records
        (4) Ascertain extent of natural dilution available

    c. Climatological factors

        (1) Wind
        (2) Temperature

4. Establishing headquarters and laboratory

    a. Headquarters readily accessible to laboratory
    b. Laboratory as close to stream as possible - permits minimum delay between sampling and analytical work
    c. Should consider location of cooperating agencies

5. Staff and Functions - Requires wide variety of technical knowledge

    a. Sanitary engineers - hydraulics, reports, sampling, interpretation of data, tie project together generally
    b. Chemists - determine sanitary quality of the waters by chemical and physical tests
    c. Bacteriologists - determine sanitary quality of the waters by bacteriological analyses
    d. Biologists - conduct special studies to determine biological conditions of stream - determine effect of pollution present on fish life, to be used as a guide in determining corrective measures
    e. Epidemiologists - conduct field studies of apparently water-borne diseases, laboratory examinations (water and stool), vital statistics
    f. Laboratory technicians, sample collectors, etc.

6. Sampling Program

    a. Establish sampling points and ranges

   (1) Sampling from boats, bridges, etc. - Points should be easy to repeat
   (2) Methods of indicating points - mileage index number
   (3) Ranges across-catch tributaries and main pollution sources, depth at midpoint, map and describe
   (4) Methods of locating points - stadimeter, boat speed

b. Frequency of sampling

   (1) Diurnal variations in dissolved oxygen
   (2) Catch batch dumps from industrial plants
   (3) Regulation of flow by dams
   (4) Minimum interval of sampling

c. Size of samples, volume depends on analyses
d. Make samples representative - stratification
e. Municipal sampling

7. Laboratory Program

   a. Determinations to be run - depends on type and extent of problem

      (1) Taste and odor - phenols and ammonia important
      (2) Domestic sewage - turbidity, B.O.D. Coliforms, etc. (tests to show organic load)
      (3) Industrial and chemical - alkalinity, pH, B.O.D., chlorides, solids, toxic substances (cyanides), metals (iron, copper, lead chromium), color.

   b. Laboratory procedures

      (1) Extensive use of standard methods - legal aspects
      (2) Justification for departure from standard methods

8. Sources of Assistance

   a. U. S. Geological Survey - Stream gaging and discharge measurements
   b. U. S. Public Health Service - Laboratory Determinations and procedures
   c. U. S. Weather Bureau - Rainfall and temperature data
   d. Pollution control agencies, public and private
   e. Miscellaneous - Publications and literature

9. Water Supply and Sewerage Survey

   a. To determine extent of use for water supply and sewage disposal
   b. To determine load discharged to stream from municipalities, institutions, private sources
   c. Shows where corrective measures are needed

10. Industrial Waste Survey

11. Hydrometric Data

    a. Obtain daily volume of stream flow during survey
    b. Establish cooperative stations with U S G S where necessary
    c. U S G S can derive discharge and stage relationships where no gaging stations are available
    d. Methods of determining velocities and flow times

        (1) Large streams - gage height records and information on channel cross sections generally available

        (2) Small streams - dyes, floats, current meters, etc.

    e. Rainfall - Aid in determining relationship between rainfall and runoff

12. Economics of Stream Pollution Control

    a. Upstream sewage treatment vs downstream water treatment
    b. Correction of condition vs replacement costs
    c. Provision of one large treatment unit vs several smaller units

13. Reports

    a. Monthly status - helps orient survey and formation of objectives
    b. Periodic progress reports - should summarize data and aid in planning future program
    c. Final - Probably only permanent record available

        (1) Sufficient time for preparation should be allowed
        (2) All data should be summarized
        (3) All pertinent facts and points should be developed in logical sequence
        (4) All conclusions should be well substantiated
        (5) Limit tabulated material to monthly or periodic averages
        (6) Diagrams and photographs good to emphasize pertinent points and details
        (7) Report proper should contain only the pertinent information
        (8) Appendices - should contain all supporting information, procedures used, and full reports on special studies
        (9) Report should be designed to contribute to the general knowledge of the subject of stream pollution

Mobile Laboratory Operation

(Russell S. Smith)

1. Definition of mobile laboratory

    A. Permanent laboratory built into movable unit.
    B. Moved to convenient location for work for period of weeks or months.
    C. Utilities readily connected or self-contained.
    D. Samples from reasonable area brought to laboratory.

2. Reasons for use of mobile laboratories

    A. Large areas to be covered.
    B. Short time spent in any given area.
    C. Lack of adequate local laboratory facilities.
    D. Difficulty and expense of establishing adequate laboratory facilities in permanent buildings for short period.

3. Description of units

    A. Original units - 1933

        (1). Described by DeMartini in Public Health Reports Reprint #2259

            a. Housetrailer shell 17' long, 6'-2" wide, 85 HP, standard coupe for tow-car.

            b. Designed for stream pollution surveys and equipped for certain restricted tests.

        (2). Changes and additions desirable.

            a. Not adequately equipped for extensive bacteriological work.

                1. Pressure cooker for autoclaving inadequate and not in conformity with Standard Methods.
                2. Hot air sterilizer inadequate.
                3. 37° incubator too small.
                4. No thermostatically controlled water bath for heating agar.
                5. Difficult to obtain distilled water of proper quality in small communities.

            b. Lack of facilities for chemical work in industrial waste and sewage treatment surveys.

                1. Lack of sufficient B.O.D. incubation space.
                2. No facilities for suspended solids, nitrites, nitrates.

c. General needs

1. Separate electrical circuits for all major units.
2. Gas for bench use and certain equipment.
3. Clothes locker.
4. Proper blinds for light control and ventilation
5. Better artificial illumination.
6. Increased space for additional workers.

B. 1944 Units

(1). Original design based on 25 ft. housetrailer shell and 3/4 ton tow truck.
(2). Unit as built.

a. Laboratory inside, 24 ft. long x 7'-2" wide. Steel frame and shell, plywood lined. Tow truck - 1 1/2 ton with 117" wheel base and vestibule panel body.
b. Additional facilities not in old units.

1. Compressed gas for benches and autoclave.
2. Larger 37° incubator.
3. Clothes locker.
4. Gas heated vertical autoclave.
5. Insulated sterilizing or drying oven with thermostat.
6. Two doors for more convenient access.
7. Fluorescent lighting.
8. Desk, typewriter and file drawer.
9. Electric wall still - 2 qt/hr cap.
10. Additional electrical circuits.
11. Additional space for larger crew.

c. Provision for other equipment

1. Muffle furnace.
2. Vacuum filtration (motor driven Nelson Hi-Vac) in refrigerating compressor cupboard with manifold on end of bench.
3. Additional B.O.D. incubator by removing 37° incubator.

d. Changes in future design

1. Three wire electrical system to balance loads.
2. Air brakes instead of electrical.
3. 1000 cfm ventilating fan in rear window.

4. Operation of 1944 units under Emergency Health and Sanitation Act

A. Purpose

(1). Milk and restaurant sanitation

(2). Other uses

- a. Emergency control of water supplies.
- b. Bacteriological surveys of shellfish areas.
- c. Bacteriological surveys of recreational waters.
- d. Special bacteriological studies.

B. Provision of site and utilities

(1). Responsibility of local community to provide.

a. Site requirements

1. Adequate size, hard surface
2. Electric power - 110 volt, single phase, 45 amp minimum capacity. (Unit has 150 ft. cable)
3. Water - adequate potable supply (unit has 100 ft. hose).
4. Drainage - sewer manhole or satisfactory ditch to handle sink wastes (unit has 50 ft. of hose)
5. Adequate toilet facilities nearby for use of crew.
6. Storage space for crates, cartons and unneeded equipment.

C. Setting up for operation

(1). Moving laboratory into position.

a. Large turning radius.

(2). Leveling laboratory.

- a. Jack screws under axle for transverse leveling.
- b. Built-in front end jack and blocking for longitudinal leveling.
- c. Connecting to water, drain and power.

  1. Special circuit and meter frequently needed. Cost $15 - $30 to install and remove.

- d. Unpacking glassware, media, chemicals, etc.

D. Operation

(1). Normal as in any permanent laboratory.
(2). Cold weather.

a. Have operated outside at temperature of 10° F.

1. Cold floors.
2. Difficulty maintaining water supply and drainage.
3. Heating.

- b. Inside location if obtainable.
- c. Winter roads.
- d. Preference for location south of snow belt.

(3). Hot weather.

a. Excessive heat in laboratory.
b. Shaded location if available.
c. Use of tarpaulin protection.
d. Aluminum paint on roof.

5. Costs - 1944 and 1945

A. Capital

(1).	Delivered cost of unit (Laboratory and Tow Truck)	$8,707
(2).	Laboratory equipment	1,448
(3).	Glassware	433
(4).	Supplies	100
(5).	Miscellaneous	167
		$10,855

B. Operating - not including salaries and living costs for personnel. Monthly cost, average of five units, January to July 1945.

(1).	Tow truck		
	Gasoline, tires, lubrication, repairs		$35
(2).	Laboratory		
	a. Glassware and supplies	30	
	b. Heat (kerosene)	4	
	c. Bottled gas	5	
	d. Power (600 kw-hrs. @ 3¢	18	57
			$92

C. Detailed cost laboratory equipment

1 -	20°C. incubator and refrigerator (custom built) (estimated, but old units used)	$400.
1 -	37°C. incubator, water jacket, double door	309.
1 -	Autoclave, vertical, gas heat	100.
1 -	Elect. sterilizing oven - precision	223.
1 -	Still, elect. 2 qt/hr capacity	55.
1 -	Balance, chemical, with weights	16.
1 -	Bath, water, serological, elect.	37.
1 -	Container, sample, restaurant	30.
1 -	Counter, colony, Quebec	30.
1 -	Kit, chlorine, residual, LaMotte, Enslow	12.
1 -	Kit, pH, LaMotte, (6.0 - 7.6)	12.
1 -	Kit, pH, S.D.C. model	52.
1 -	Titration lamps, stand and burette holder	17.
2 -	Stools, laboratory	15.
20 -	Cans, petri dish, copper	45.
7 -	Cans, pipette, copper, square	15.
	All other, such as wire baskets, pots, ring stands, aprons, funnel racks, etc.	80.
		$1,448.

6. Other possible uses of unit

A. Stream surveys

(1). Can cover approximately 100 miles of stream from single location.
(2). Smaller area if tributaries are also sampled.
(3). Area covered depends on time required to get first sample to laboratory.

B. Sewage plant studies

(1). Special investigations where plant laboratory is non-existent or inadequate.

C. Industrial waste studies

(1). Unusual wastes.
(2). Pilot plant laboratory for waste treatment.
(3). Variety of wastes from major industries in connection with stream pollutional control program.

D. Control of water treatment and distribution system in disaster areas.
E. Milk shed sanitation control in rural areas without adequate local laboratories.
F. Bacteriological work in connection with epidemiological surveys.
G. Engineering field office and biological laboratory in connection with stream surveys.
H. Any other purpose where it is desired to establish a well equipped small bacteriological or chemical laboratory for a limited period.